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ATMOSPHERIC FUNGI

[Following is a translation of a pamphlet by Jose M. Quintero Fossas in the Spanish-language Bulletin 178 Hongos Atmosfericos (English version above), University of Puerto Rico, 1964, pages 1-67.]

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ATMOSPHERIC FUNGI

(Considerations of Climatological and Mycological Factors in the Caribbean Area and Importance of Their Allergenic Role in Respiratory Allergies)

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INTRODUCTION

Climatic conditions in the tropics and in the temperate zones make the content of micro-organisms in the air different in quality and quantity.

Conditions of relative stability in humidity and temperature throughout the year make the content of fungi in the air in the Caribbean high and constant, and also have similar influence on other organisms that affect the human being. Because of this, at least a 15 or 20 percent of those suffering from allergies of the breathing apparatus have developed responsiveness to them. This estimation, although conservative enough, and based on thousands of cases, helps explain the importance of studying and discovering the causes.

The role played by the pollen of plants is the inverse, since the degree of responsiveness to them is lesser due to the atmospheric conditions and insular flora adverse to causing a high pollen content in the air. The frequency of rainfall, high hygrometric readings, and the nearly total absence of well-defined seasons have determined the adaptation of many plants to such meteorological conditions by suppressing the urgency for pollenization. The liberation of great quantities of pollen in a short period of time, has been replaced, therefore, by a lesser but lengthier liberation.

Here, then, we shall deal mainly with the part played by airborne fungi in the genesis of respiratory allergies in this zone, studying variations in fungi content both quantitatively and qualitatively. Some of these elements have been found to follow a certain pattern or curve which would account for their greater importance at a particular moment.

Although it is expected that in summer there will be a greater amount because of the concurrence of factors aiding multiplication, many spores in certain winters loosen more easily due to drought. Contact with material containing parasites, clothing, and articles that have long been stored in closets or other such places are likewise other means of propagation. In closed structures such as houses or warehouses, the summer humidity constitutes another favorable agent. The same is true in the presence of great amounts of material susceptible to fungi parasitization at a time other than in summer, as in the case of refuse from certain industries which is later to be used as fertilizers.

Our study is based on careful observation of air samples taken for almost one year in Puerto Rico, and compared with similar studies conducted in Cuba in previous years. Knowledge of both such Caribbean areas will result in richer conclusions regarding their mycological content

and in better judgment of their individual variations.

We have studied different zones having temperature and humidity conditions bearing on the content of atmospheric fungi. Part of this study took place in Cuba, at one end, so to speak, of the Caribbean, but closer to the continent; the other part took place in Puerto Rico, at the opposite end and away from the continent, under conditions which, that though they may be thought of as identical, are not so.

All the Caribbean islands immediately below the Tropic of Cancer, slightly north of Havana, have a tropical climate. These islands are partly flat and partly mountainous with a predominance of plains, excepting Puerto Rico where the inverse is true, and where, because of rainfall from local cloud masses and the Trade Winds blowing against the mountains, humidity is higher. This characteristic in particular favors to a high degree a greater abundance of fungi and greater growth of plants in Puerto Rico and surrounding islands.

The distribution of rainfall at both extremes of the Caribbean also differs in characteristics. In Cuba, for instance, a humid season extends from May to November, and one of relative dryness, which is on occasion marked, from December to May. This division is less apparent in the case of Puerto Rico, where there is a rainy "winter." This latter condition, together with a greater amount of parasitable material, maintains a high fungi content, with some species reaching their apogee from December to March or April.

The mycotic air content in the United States is different, having its own characteristics; and from the point of view of allergies, this facilitates the knowledge and prediction of when the content will be low. In the Caribbean islands, on the other hand, while seasonal and individual variations tend to modify the type of existing species, they have no great influence on the quantitative content.

It must be pointed out that the introduction of fungi into Cuba, and in turn from Cuba to the Continent, is aided by the proximity of Cuba to the United States mainland, as well as by north and northwest winds that prevail in the winter months and which are superimposed to the regular Trade Winds. In Puerto Rico, by contrast, the incidence of northern winds is much less, and they come from the sea. But in spite of this, fungi are transported over great distances through the air and are deposited on these regions by the

rains. We know that fungus spores can be found as far away as the North Pole, as well as in the tropics or in the middle of the ocean, far away from continents.

It is important to make a detailed consideration of the various climatic factors of the different zones under study, in order to relate them with the individual mycological characteristics in spite of the latter being already interrelated.

As already stated, one of the purposes of this work is to make a comparative study of the allergenic mycological flora of Puerto Rico and neighboring islands with that of the western zones and their islands, in order to find out the possible difference between those islands close to the continent and those of the eastern end, such as Puerto Rico and Martinique. The latter has been included as a result of air samples taken during a visit to it. Once in possession of data of the northwest, northeast and southeast Caribbean zones, it is easier to understand the differences existing between them. Greater importance shall be then given to data relating to Puerto Rico and Cuba, since the bulk of the investigation was conducted in these places.

The Puerto Rican Climate

Puerto Rico is situated at a latitude of 18 and 18.30' N., that is, within the traditional torrid or tropical zone. Thus it is situated within that part of the globe receiving the maximum sunlight, namely between the tropics of Cancer and Capricorn. In this zone, the noonday sun is nearly always close to the zenith, and its rays fall almost vertically with great heating power.

Since Puerto Rico is a small island, the sea has a prevailing influence. This maritime influence acts as moderator, since water takes longer to heat up and to cool off than the earth; thus the sea helps keep the island warm in winter and cooler in summer. Because of this, temperatures never reach extremes of heat and cold observable in the interior of continents and, to a lesser degree, in great islands. By the same token, maximum and minimum temperature readings do not coincide with the days of greatest and least sunlight; instead there is a lagging from one to two months in warming up and cooling off. Therefore the maximum heat and cold are reached at the beginning of August and February respectively, instead of those dates marking the beginning of the cooling

off and warming up processes. Such is the alteration suffered by the island in these two months.

Puerto Rico lies towards the east and towards the middle section of the great continental mass of Americas and to the west of the North Atlantic. This position, in combination with latitude, affects the wind regimen and places the island within the ascending arm of the great North Atlantic marine current. This North Equatorial Current originates on the African coast, close to South America; it passes through the Antilles, penetrates the Gulf of Mexico, exits by the northeast sector, and reaches the European shores by again traversing the Atlantic. From there it turns south once more, along the western coasts of Europe and North Africa to complete the circuit.

The ascending arm of this current, known as the Northern Equatorial Current, is warm and humid, and helps intensify this characteristic of our climate. On account of the vicinity of these masses of warm water flowing along the coasts with the Northern Equatorial Current, the Puerto Rican climate, and in general that of the Antilles, is somewhat warmer than it would otherwise be. On the surface of these waters, evaporation is simplified since no great amount of sun is required to heat them, resulting in more cloudy skies for all this part of the Antilles, which is in turn translated into more frequent rainfall.

This data, lifted from very interesting local studies by Dr. Rafael Pico, and from others on the geography of Puerto Rico, avails us better knowledge of local conditions. The winds blow mainly in the leeward zone, that is from the East.

Influence of the Trade Winds

Puerto Rico's latitude places it within the influence of the Trade Winds, blowing in this region from the east throughout most of the year. These winds in general have a drying effect, absorbing the humidity of the places along their path, and without tending to produce precipitation except when they encounter mountains and are forced to rise, cool off, and release part of the humidity, what is known as orographic or mountain rain. This is what takes place in Puerto Rico, and similarly in other islands with mountain regions.

The Trade Winds are felt more in the northern, eastern and southern coasts, and on the windward side of the mountains. The Western coast and the windward side of the mountains are better sheltered and suffer from them less. The same is true of interior valleys that are well protected by the mountains.

According to meteorological data from San Juan, the Trade Winds blow from the east about 50 percent of the time, 10 percent from the northeast, and 19 percent from the southeast. In winter these winds have a tendency to blow from the northeast, and in summer from the southeast, but always with a predominance of those from the east. The two periods of greatest velocity and force are winter and the month of July. On certain days, this influences fungus content, the latter being directly proportional to wind intensity.

Sea breezes occur during the day, because at that time the land warms up more quickly than the sea. This causes the air to warm up and ascend, leaving a vacuum to be occupied by the cooler air above the sea near the coast. This displacement of air also works inversely: the land cools off more rapidly than the sea, and the sea air, then warmer, ascends; the air above land moves in to take up the vacuum, resulting in a slight dawn wind which we call land breeze or "terral," and which is much more gentle than the sea breeze.

The sea breezes tend to have a cooling effect on the climate, smoothing out extremes in temperature and making the summer heat bearable, especially in the shade. Although these Trade Winds blow in the same direction and with equal consistency throughout the year, the breezes change direction twice every twenty-four hours. They have quite variable intensity, many intervals of tranquillity, and two larger periods of calm. Generally, the Trade Winds are felt, although weakly, since as a rule their intensity is greater at greater heights than the breezes; they are hardly experienced near the coast. The breeze is a low wind whose main effect is on temperature airing and cooling coastal atmospheres. Since Puerto Rico is a small island, it can be said that the effects of the sea breeze are felt in at least one third of its territory.

It is important to know such characteristics, since fungus curves are influenced by them. This will explain, for instance, the difference between quality and quantity of fungi found during the day, and what takes place after

6:00 p.m. when the breeze changes. Sometimes, variations are quite evident, judging from plates exposed at both these times. Generally, Dematiacea species predominate during the day, together with others belonging to the Monolial group.

Valley and Mountain Breezes

Another type of breeze, of a very local character, is the valley and mountain breeze. The mountainsides, exposed to the sunrays from the beginning of the day, warm up quickly, while the valley at their foot remains relatively cool. As a consequence, a high pressure area is formed in the valley, and a rarified or low pressure area is formed in the mountain, the cool air rising towards the mountain along its sides. The inverse is true at night; the mountain radiates its heat more rapidly than the valley, and the high and low pressure areas are inverted; the cool air from the mountain descends onto the valley. In both cases, it results in a tendency to equate the temperature of the mountain and that of the valley.

This is a pronounced phenomenon in some valleys of the Cordillera Central [Central Mountain Range]. Along the lesser and protected valleys that descend from the Cordillera Central in the south and, probably, the west of Puerto Rico, one can easily feel the slight wind that rises from lower parts of the valley in the early morning hours, and which descends more rupty from higher to lower ground at nightfall.

We have found a somewhat different fungus content in the central zones; that is, while we found *Monilia sitophilae* fungi in the coastal and sugar cane zones, *Cladosporium* and diverse species of the *Dematiaceae* family predominated in the center of the Isle.

Characteristics of the Rain

Rain in Puerto Rico is due to four main factors: relief or topography, convection, Trade Wind waves, and the hurricanes.

The relief causes what is known as orographic rain. This rain is found particularly in the windward parts of the mountain areas and corresponding foothills; once the mountain forces the humid wind to rise, the humidity condenses and comes down as rain. The rain falls not only on

the mountain itself but on the foothill on the windward side as well. The opposite side, on the other hand, will not normally receive rain from such winds.

Convectional rain is due directly to heat. When the air near the ground is very warm, which occurs during the hours of major sunlight, it becomes rarified and rises, probably containing considerable humidity caused by the fact that the sunlight has evaporated the water. While rising, the air continues to cool, and the humidity continues to condense to produce those midday clouds common especially during the summer, resembling huge masses of cotton tufts, from which some emerge like very high towers several kilometers in height. These towers are made by hot air currents rising inside the clouds. When at last those clouds turn into rain, it is often a downpour of short duration.

The changes in humidity have a direct effect on the falling off of fungus spores, since the greater their density or weight, the greater will be the speed of their fall. This shall be dealt with later by application of the laws of body fall to the different spherical spores. This should not be confused with the spores being driven by the rain. Still, it is interesting to point out that a few years ago we made some studies on fungus content within clouds as compared with the air outside them, and were unable to find any marked difference in favor of the former as we had suspected.

Convectional rain and orographic rain are similar in that precipitation is caused by the cooling off of ascending, humid air. The difference is that in convectional rain the air ascends as a result of heat, while in orographic rain it ascends because it moves over a mountain-top. In both instances the result is the same; but in the case of orographic rain, the cloud producing the rain is nearly always lying low over the land, and the rain is one of droplets or rather a long and intermittent drizzle. In convectional rain, the cloud is at a great height and the falling drops unite with one another, and reach the ground in continuous, large drops as a short duration downpour, usually late in the morning or early in the afternoon. Both have the same effect on the temporary cleanliness of the air. Although the fungus content drops during rain, it goes up soon as the rain stops.

In the Trade Wind waves as was indicated, there is, a part -- the one east of the axis -- where the air is rising. If this air contains humidity, which is normal, the

humidity will be condensed, forming clouds that end up as rain. Rain from Trade Wind waves is characterized by a long duration of wet weather, quite often lasting a whole day, and on occasion two and three. According to McDowell, about one third of Caribbean rainfall is due to these waves.

Two Types of Seasons

Rainfall has certain evident characteristics. Everywhere there is a rainy part of the year -- the rainy or humid season -- and another less so -- the dry season.

The dry season can last from 4 to 5 months. The first 4 months of the year are always relatively dry, excepting the west coast and the western and central portion of the mountains, where April is already humid. In these regions and in the southern coast, December is also dry, resulting then in 4 months of little rain, and even in five very dry ones for the southern coast.

The remainder of the year can be rainy, with some differences among the local measure of rainfall. The rainy season has two maximum readings for most of the island, except for the west coast where there is only one, and in the Luquillo Mountains [Sierra de Luquillo], where there are three. The first top reading usually takes place at the beginning of the rainy season, in May or June; the second takes place in November for those areas where December is humid, or in September or October where December is dry. In the west coast, the only maximum reading takes place in August; in the Luquillo Mountains, the three maximum readings occur in May, July and November.

The fact that the dry season coincides with the coldest months is explained by the fact that at that time there is less evaporation, and thus less humidity to produce rain clouds in the atmosphere. The tendency in the northern and eastern parts of the island to have a humid December is due perhaps to the predominance at that time of Trade Winds from the northeast that still carry enough humidity. By January and February, even though they still blow from the northeast, they no longer carry enough humidity, and little rain results.

The air samples taken in order to find out the number of colonies differs if the sample is taken, 1, without rain and with stability in temperature and wind; 2, with rain, during the first few minutes; or 3, with rain, after it has

been falling for a while. In the first case, we will find the fungi prevailing at that time. In the second, a series of different fungi will appear, originating on the surface of the earth and on plants that will release spores on being struck by the first rain drops. In the third instance, the count will go down because the spores are being dragged by the rain. This explains why some patients experience nasal discomfort and sneezing when it begins to rain, but get over it if the rains continue for a longer period.

Puerto Rico's temperature is fairly constant throughout the year resulting in little variation in atmospheric conditions in the different seasons. The latter are hardly distinct in the Island (chart 1).

Other factors related to the mycotic content in the island should also be considered. This is the result of the flora of the different regions, especially of the different harvests, their parasitation, and the use of industrial refuse. This is why the coastal zones, where most of the sugar industry is located, have a particularly higher content of fungi during the milling months, as we shall see later when we deal with this in greater detail. The same thing happens in areas where other minor harvests take place, especially tomatoes, where certain fungi parasitation, in this case pathogenic, takes place, such as Cladosporium fulvum and Collectotrichum gloeosporioides (in oranges). Of course, we must not confuse parasitation of a harvest by pathogenic fungi with fungi whose spores are easily freed and constitute part of the air's allergenic content. Most of the latter are saprophytic.

The central zone, which could be defined as that of tobacco and coffee, has more defined characteristics. Besides climatic and agricultural characteristics, one must consider population distribution in Puerto Rico, since in the last instance it will be the one exposed to the effects of the different fungus spores. It is estimated that 10 percent of it, that is 275.000 Puerto Ricans, have or have had some allergic manifestation in their lives at one time or another, either major or minor in degree.

We are still unable to learn in what proportion of them the respiratory problems are allergenic in nature. There may be some 15 or 20 percent of the population that is sensitive to fungi, where it results in bronchial asthma, allergic rhinitis, or any other manifestation caused by such elements in the air, indicating the importance of its clinical and mycological study.

Naturally, the most highly populated areas merit special attention, because of population concentration and on account of other factors related to the different industries. Some of the latter, such as leather and all others dealing with products of a vegetal nature, facilitate parasitization and hence sensitivization of the personnel.

The type and ventilation of the dwellings, their site, the furniture, and the parasitable material in them deserve special consideration, as it is known that the fungi content in dwellings is generally higher than that of the air outside. Warehouses also require special consideration.

The Cuban Climate

The climatic conditions of this island, lying on the western portion of the Caribbean Sea, deserve to be studied with interest, although in less detail than in the case of Puerto Rico. Being the largest of the islands, and also the closest to continental America, the mass of its atmospheric air is somewhat different. It has a surface of 45,000 sq. miles, a longitude of 720 miles, and an average width of only 60 miles. The maximum width is 120 and the minimum 22. Cuba lies 90 miles from Key West; 140 miles from Florida; 125 from Yucatan; 94 from Jamaica and 50 from Haiti. It is not a mountainous island, except at the extremes and in one central area. The highest point of the oriental province is 8,200 feet, but most of the mountains are less than half of that. The island is surrounded by large beaches and bays and a considerable number of keys and small islands. Although lying south of the Tropic of Cancer, it is close enough to the temperate zone to have a relatively moderate temperature throughout the year. Summers are hot, but the heat is not excessive, as is true of more northerly places. Temperature seldom exceeds 90°F, the average summer reading being around 80°F. Winters are cool, with temperature of 65 to 70°F. Rarely does the temperature dip below 40 or 50°F, January and February being the coldest months, although there are days in them when the temperature may go up to 80.

Temperature has no major influence on plant distribution; it is, however, an important factor in pollenization. It also has a definite influence on grasses that dry up in winter and bloom abundantly during the rest of the year.

Strictly speaking, there are only two seasons, the rainy one commencing in May and lasting until the end of

Chart 1

Monthly Temperature Averages (F) in San Juan, Puerto Rico

1) Temperatura	2) Enero	3) Febrero	4) Marzo	5) Abril	6) Mayo	7) Junio	8) Julio	9) Agosto	10) Septiembre	11) Octubre	12) Noviembre	13) Diciembre
14) Mínima	70	69	70	71	73	74	75	75	75	75	73	71
15) Máxima	80	80	81	82	84	85	85	85	86	86	84	81
16) Promedio	75	75	75	77	79	80	80	81	81	80	79	76

Chart 2

Monthly Temperature Averages for Cuba (F.)

1) Temperatura	2) Enero	3) Febrero	4) Marzo	5) Abril	6) Mayo	7) Junio	8) Julio	9) Agosto	10) Septiembre	11) Octubre	12) Noviembre	13) Diciembre
14) Mínima	65	65	67	69	72	74	74	75	74	73	69	67
15) Máxima	79	79	81	84	86	88	89	89	88	85	81	80
16) Promedio	72	72	74	76	84	81	82	82	81	79	75	74

[Legend]: 1) Temperature; 2) January; 3) February; 4) March; 5) April; 6) May; 7) June; 8) July; 9) August; 10) September; 11) October; 12) November; 13) December; 14) Maximum; 15) Minimum; 16) Average.

October, and the dry one extending from November to May. Both have a direct effect on plant and plant reproduction, although never as markedly as in the Temperate Zones. Cuban plants, therefore, have ample time in which to carry on their pollenization, rather than being forced to do it in a fixed and hasty manner. This accounts for the small quantity of pollen in the air, with a few exceptions in certain periods, especially in the months of January and February, when certain plants with precise characteristics bloom; and in summer when grasses predominate and the content hits top marks. Humidity is high, with an average of about 75 percent.

Fungi tend to reproduce easily as in the case of Puerto Rico, although humidity is lesser. There are small differences peculiar to certain species due to local conditions.

Population concentration is also different, Puerto Rico having 643 persons per sq. mile, and Cuba only 120. In Cuba the population of the country, that is $1\frac{1}{2}$ millions out of $7\frac{1}{2}$. In Puerto Rico, San Juan's metropolitan area with 600,000 inhabitants is in somewhat a similar proportion, since the island has $2\frac{3}{4}$ millions. But the main difference is in the interior where the number of inhabitants per sq. mile in Puerto Rico is over 5 times higher. Hence the correlation between the mycotic content of the air and the number of susceptible persons is more significant in Puerto Rico. If, for the purposes of such correlation, we consider the following factors: 1, number of spores per cubic yard of air; 2, cubic yard consumption per person in 24 hours; and 3, number of inhabitants, we could have, as we shall see, a means of calculating the possibility of more allergenization in Puerto Rico resulting from a higher concentration, and thence a greater amount of fungi per yard of air consumed. Because two of the factors are variable and the third one greater, it is logical that in a larger population per sq. mile with access to the same polluted air, there will be a greater amount of illness than in one where the population is less numerous.

Although we have made a study of air samples from Sr. Croix, the Virgin Islands, and Martinique to the south, the study was much too brief to be considered a serious reference. In Puerto Rico, instead, the study took one year; it took several years in Cuba. But we believed that the characteristics of the above-mentioned islands are very similar to Puerto Rico's. In both, meteorological conditions are similar, especially in the Virgin Islands. Thus, in St. Thomas as well as in St. Croix, the annual average temperature is as shown in Chart 3.

Chart 3
Monthly Temperature Averages in the Virgin Islands (St. Thomas;
St. Croix)

1) Temperatura	2) Enero	3) Febrero	4) Marzo	5) Abril	6) Mayo	7) Junio	8) Julio	9) Agosto	10) Septiembre	11) Octubre	12) Noviembre	13) Diciembre
14) Mínima	69	70	70	71	74	75	76	75	74	74	73	70
15) Máxima	84	84	84	85	86	87	88	89	88	87	85	84
16) Promedio	77	77	77	78	80	81	82	82	81	81	79	77

Chart 4

Monthly Temperature Averages in Fort de France, Martinique

1) Temperatura	2) Enero	3) Febrero	4) Marzo	5) Abril	6) Mayo	7) Junio	8) Julio	9) Agosto	10) Septiembre	11) Octubre	12) Noviembre	13) Diciembre
14) Mínima	69	69	69	71	73	74	74	74	74	73	72	70
15) Máxima	83	84	85	86	86	86	86	87	88	87	86	84
16) Promedio	76	76	77	79	80	80	80	81	81	80	79	77

[Legend]: 1) Temperature; 2) January; 3) February; 4) March; 5) April; 6) May; 7) June; 8) July; 9) August; 10) September; 11) October; 12) November; 13) December; 14) Maximum; 15) Minimum; 16) Average.

Chart 4 shows the temperature readings of Martinique.

Here is a resume of temperature differences in the four main Caribbean divisions in the hottest and coldest months:

<u>Place</u>	<u>Average Min. (F)</u>	<u>Average Max. (F)</u>
Cuba	72 Jan and Feb	81-4 May to Sept.
Puerto Rico	75 Jan to Mar	80-1 Jun to Oct
Virgin Islands	77 Dec to Mar	80-2 May to Oct
Martinique	76 Jan and Feb	80-1 May to Oct

The amount of rain and the number of rainy days in the year also vary among them: Cuba, 121 days per year; Virgin Islands, 205; Puerto Rico, 214; Martinique, 223. These figures are comparative and have been gathered on the coast areas.

The vegetation and mycologic content of the air is very similar in the different islands, Cuba being the only one having a greater variety of plant species because of its size and proximity to the Continent. Diversification, progress, agricultural industrialization, and development are greater in Cuba and in Puerto Rico; the same goes for general industrialization in both countries. Dwellings and living conditions therefore, are, also different. All this has a greater or lesser effect on the particular characteristics of sensitivity to microorganisms in the air for each one. Another aspect of interest is the diversity in the ethnic content of the different zones, and the allergenic reaction peculiar to each group.

Method of Study Plate Culture

In order to be able to arrive at a meaningful comparison of figures and data obtained at both ends of the Caribbean, in Puerto Rico we used the method of exposing of Petri dishes for 12 minutes, since the method of small plates (or sheets) would not enable us to identify all spores, and many would go ignored. When we get out of the sphere of dematiaceae and like groups, it is difficult to correlate the conidia of the different Aspergillus, Penicillium, Monilia, etc. In certain instances, we also made use of 3- and 10-minute plates.

There is also an interrelationship between the amount found in dishes during 12 minutes and the amount of spores during the 24 hours of the day obtained in the Durham apparatus, using the small plate method; but since the number of colonies was so large, we decided to use smaller time units in comparative studies.

It should be indicated that although the great majority of the dishes were exposed on the terrace of the Pathology Department in the Agricultural Experimental Station at Rio Piedras, some were developed in the building's lower floor or other points of the Station in order to compare results. Dishes for reference were also exposed in Ponce and in different locations of the central mountain range.

Notice that the colony sowing method is subject to natural error derived from variable atmospheric conditions, whereby a sample taken in a particular season may not contain the same fungi variety found there in previous days nor in subsequent days. In order to know more about the causes of such variability, we exposed from 5 to 6 dishes at 3-minute intervals. All were open and closed at the same time, and the same happened with the identification of the colonies some four or five days later.

Importance of Multiple Sowings

Results and comparisons of the dish series were highly significant, as can be appreciated in the chart appearing in another page of this study. In some instances, the qualitative difference was great, suggesting that one only dish exposed to mycological content during 12 minutes is never significant. It will therefore be necessary in the future, that studies conducted with the help of automatic equipment be done in series.

Since the daytime curves on fungi variety are subject to a certain margin of error, we recommend that more than one dish be exposed in order to find out what is happening in the air. Also the velocity and direction of the wind must be taken into consideration at each moment. In San Juan, the wind varies in the manner explained in the above-mentioned chart.

As was to be expected three of the five dishes exposed were quite similar; a fourth one was somewhat different, and still another was completely different. We assign

the differences to an air mixture that was not very homogeneous, and to the fact that there were spots within the circulating air mass, that is places where the fungi in transit differed from the rest of the samples.

The results indicate that no curves can be plotted on the basis of just one sample taken once a day on the same spot.

The use of automatic equipment would reduce the margin of error, and would result in curves of easy enough interpretation. Of course, what has been done has no diminishing results on the value of the investigation regarding: 1, the abundance of fungi in one zone as compared to another; 2, the frequency of occurrence of the different species, and 3, the presence of the zone's most common fungi. Besides, this is the method employed in other countries, especially in North America. We are not against the use of small plates, as long as their use is confined to the study of a few fungi.

The result was always related to certain conditions accounting for the diminution of colonies during rain, that is when the air reaching the dishes had passed through a liquid curtain dragging the fungi towards the soil, although in other instances we suspected other secondary factors as responsible for certain anomalies.

For instance, in some dishes exposed for 12 minutes, the colonies numbered 20, increasing considerably during the first few minutes of rain. At other times, immediately after the rain had ceased, the count climbed abruptly. The explanation might be found in the following reasons:

In the first case, during "pre-rain," the first drops reaching the dry earth near the dish freed many fungi unequally contained in wet soil, as if the dust spot was stirred by the impact of the drops. The second case can be explained by the fact that, since rain in the tropics is of a typically local character, when a cloud discharges its contents, a mass of new air will come to replace the one descending, and new air currents will blow, bringing fungi from other atmospheric strata.

One is able to understand the heterogeneity of the aerial mixture by realizing how many elements are at play, including the ascending and descending currents due to the differences in local terrestrial temperatures. Series automatic takes of air volumes at different hours would therefore

offer a solution to the problem. There are some apparatuses to obtain such samples, although they are still imperfect.

In Cuba, Cadrecha took samples by means of a sealed box with a plate in its lower part that opened quickly to take a volume of air that would sediment its spores on the plate. This is the case of a predetermined static volume.

In order to be able to compare the fungus curves at, we decided for the purposes of this investigation to rely on the Petri dish method, related to the small plates method on certain occasions, and for which, besides having taken many samples to know the fungi variety of Puerto Rico, only one was used, exposed in the morning at 10 a.m., in order to compare the curve.

We must state that the different curves obtained through the years do not follow the same pattern as in the United States, where they climb markedly in summer, drop to a minimum at the beginning of the cold weather, and drop still lower when snow comes. In our regions, the curves show different peaks during the year, and, except for higher incidence of some fungi in some periods than in others, they do not follow a fixed pattern.

In Cuba and Puerto Rico, where atmospheric conditions are more stable and propitious, the curves are perennially high and without those pronounced high and low fluctuations that occur in other places, except when proximity to certain harvests or the abundance of parasitable material may increase the amount of a particular fungus, although without great alteration in the annual curve.

Influence on the Atmospheric Factors

It should be pointed out that during the day the fungi proportion or curve is affected by a series of variables independently of its more or less homogenous concentration in the atmosphere. This is due to these different factors:

1. Wind velocity: the higher the velocity, the greater the amount of colonies in the dish.
2. Wind direction: this only affects fungi variety and not quantity. This factor has repeatedly surprised us when comparing the dishes exposed

during a day having northeast winds with those exposed during a night having southeast winds. As a rule, accounting for natural exceptions, the content of night fungi is different from the day ones (Naturally, I am referring to the air outside and in motion). Also fungi of whitish colonies tend to predominate at night.

3. Temperature: this is generally in direct relationship to the amount of fungi.
4. Barometric pressure: A general inverse relationship with it exists.

In San Juan, the wind system on the wind compass is the one indicated in the accompanying figure.

...to see the variations that take place in the 24 hours of the day (Fig. 1). We assign importance to consideration of this aspect in the particular case of fungi from definite zones where it may be considered that a large source exists.

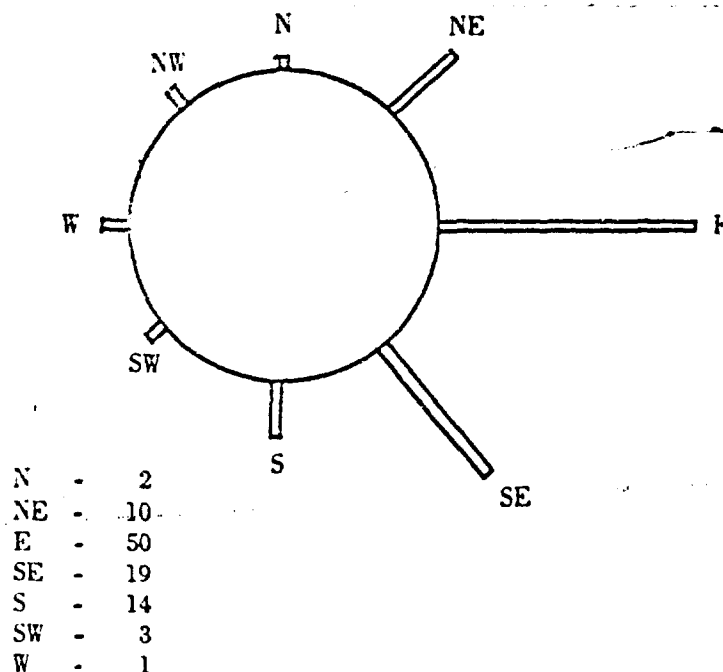


Fig. 1. (Numbers above are percentages) San Juan's Wind Compass. We can see that the majority of fungi from a particular zone originate with or are transported by air currents from the east and northeast during the day, and from the southeast at night.

The influence of the degree of clarity of the sky in our climates does not seem to be of real importance, since it quickly changes in a few hours.

Fungi are only a part of that large conglomerate of living organisms which constitute part of our soil and which contaminate our immediate atmosphere. Their number in nature is enormous and individual. It is difficult to study them because of their diversity. Because of this, however, they are an increasingly fascinating subject. Their functions, some beneficial, and their scientific utility on the one hand are, from other points of view, harmful. Let us cite the parasitism of the human being in certain cases, and, more so, the production of allergic manifestations in man due to respiratory fungi. Their action is otherwise harmful in the infestation and destruction of large amounts of materials and food. Yet, this very same action permits the elimination of much waste matter.

Description of Fungi

From the point of view of this investigation and on account of its limitations, we shall deal mainly with the study of the asexually-reproduced spores of one series of fungi, especially the Fungi Imperfecti, since they constitute the most important group for our purposes. We will also study the phicomyceti group.

The Fungi Imperfecti may be divided into four "groups" in accordance with the structure of the apparatus producing spores. These are as follows:

1. Sphaeropsidali - Conidia is produced in picnidiae.
2. Melanconiali - Conidia is produced in an acervuli.
3. Moniliai - Conidia is produced in conodiofori, but never in picnidiae or acervulii. On occasions, it is produced by off-shoots or by breakage of the hifa. The Moniliai group will be the most important in our study.
4. Here Conidia are absent, and the fungi are temporarily called Mycelia sterila.

Another class that should be studied is the basidiomyceti, and in them the groups of the uredinali and ustilaginalli, which we have not dealt with because at the moment we are only interested in sowable fungi. Therefore, the blights

and mildews of the air will be the subject of another writing at another date, since as their importance in this respect is insignificant.

In order to classify those fungi appearing in the air with more frequency during our observations, we have classified and identified them on the basis of their different families, tribes, genera and species. Naturally we have only included the genera found in the air of Puerto Rico (Chart 5).

In order to facilitate and accelerate their identification, we have proceeded to divide them as to spore type and as to the presence of one or more cell and septa or internal partitions, segregating them as shown on Chart 6.

On the following page is a list of fungi genera and species most commonly found in the air samples of Puerto Rico, together with corresponding spore sizes (micras).

Especies	Micras
<i>Blakeslea trispora</i>	5-8 x 10.5
<i>Cunninghamella</i> sp.	12 x 9
<i>Aspergillus parasiticus</i>	2.7 x 3.4
<i>A. nidulans</i>	2.5 x 3.5
<i>A. niger</i>	2.5 x 4
<i>A. luchuensis</i>	4.5
<i>A. tamarii</i>	3.5 x 7
<i>A. terreus</i>	2.2 x 3
<i>A. flacus-oryzae</i> group	2 x 3 y 4 x 5
<i>Periconia</i> sp.	6 x 8
<i>Nigrospora spherica</i>	11 x 14
<i>Verticillium dactyloides</i>	4-6 x 3
<i>Cryptosporium</i> sp.	7 x 2.5
<i>Trichoderma viride</i>	3.8 x 5
<i>Cladosporium cladosporioides</i>	10-14 x 4
<i>Cephalosporium acremonium</i>	4 x 1.5
<i>Curtularia</i> sp.	23 x 27 y 9 x 10
<i>Helminthosporium sativum</i>	60-90 x 12
<i>H. anomalum</i>	45 x 90
<i>Helminthosporium</i> sp.	48 x 75
<i>Fusarium</i> sp.	20-46 x 3
<i>F. nivale</i>	19 x 30
<i>Fusidium</i> sp.	10 x 3.5
<i>Pestalotia</i> sp.	40 x 60
<i>Penicillium nigricans</i>	3-3.5
<i>P. lanuginosum</i>	2.4 x 3
<i>P. decumbens</i>	2.5 x 3
<i>Penicillium</i> sp.	2.3 x 2.8
<i>Rhizopus nigricans</i>	10 x 8
<i>Mucor</i>	4 x 6
<i>Cunninghamella</i>	6 x 8
<i>Sepedonium</i>	14-16
<i>Stenophyllum</i>	30-40

Chart 5
Classification of Fungi Most Commonly Found in the Air

1) Clase	2) Orden	3) Familia	4) Sección	5) Tribu	6) Género
Ficomicetos	Mucorales	Mucoraceae			Mucor Rhizopus
Ascomicetos	Sphaeriales	Chaetophoraceae			Cunninghamella
	Sphaeropsidales	Chaetomiaceae			Chaetomium
	Melanconiales	Sphaerotiellaceae			Phoma
	Moniliales	Melanconiaceae			7) Colletotrichum ¹ Monilia
		Moniliaceae			Cephalosporium Trichoderma
					Aspergillus
			Hyalosporae	Oosporae	
				Cephalosporiaceae	
				Aspergillaceae	
				Botrythaceae	
				Verticillaceae	
				Actinomycetaceae	
				Torulaceae	
			Phaeosporae	Monotosporaceae	
		Dematiaceae		Dematiaceae	
				Chaetochaceae	
			Phaeophragmataceae	Helminthosporiaceae	
				Acrotheciaceae	
					Helminthosporium Curvularia
					Sporobolomyces Acrothecium
					Fusarium
					Cratium
Fungi imperfecti		Tuberculariaceae Stilbaceae	Hyalophragmataceae		

[Legend]: 1) class; 2) Order; 3) Family; 4) Section;
5) Tribe; 6) Genera; 7) Only found in parasitized
orange trees. Because of its characteristics, not be-
lieved to be in the air in sufficient quantity.

Chart 6

Classification by Conidia

1) Clase	2) Color de la Conidia	3) División por Sección de Familia
4) <i>Americosporae</i> (1 célula)	<i>Hyalosporae</i> (conidia hialina)	<i>Monilia</i> <i>Cephalosporium</i> <i>Trichoderma</i> <i>Aspergillus</i> <i>Penicillium</i> <i>Sapronium</i> <i>Botrytis</i> <i>Verticillium</i> <i>Spicaria</i> <i>Actinomyces</i>
5) <i>Didymosporae</i> (2 células)	5) <i>Phaeosporae</i> (Conidia bien coloreada)	<i>Torula</i> <i>Nigrospora</i> <i>Hormodendrum</i>
6) <i>Phragmosporae</i> (3 ó más células)	3) <i>Phaeodidymae</i> (conidia bien coloreada)	<i>Cladosporium</i>
	3) <i>Phaeophragmiae</i> (conidia bien coloreada)	<i>Helminthosporium</i> <i>Acrothecium</i> <i>Curcularia</i>
	2) <i>Hyalosphragmiae</i> (conidia no coloreada)	<i>Fusarium</i>
7) <i>Dictyosporae</i> (septos transversales y longitudinales)	<i>Phaeodictyae</i> 7) (septos transversales y longitudinales)	<i>Stemphylium</i> <i>Alternaria</i>

[Legend]: 1) Class; 2) Color of the Conidia; 3) Division by Family Section; 4) 1 cell; 5) 2 cells; 6) 3 or more cells; 7) transversal and longitudinal septa; 8) well-colored Conidia; 9) non-colored Conidia.

These fungi, and also the ones included in the previous charts offered here, are the group most commonly found in daily air samples. As we have already said, we want to point out that curves based on only one 12-minute samples are not representative of the true aerial content. In order to prove this, we exposed separate plates 3 meters apart as follows:

Plate 1: 1 *Monilia sitophila* colony, 3 small *Cladosporia* colonies, and 3 *Aspergilli* sp. colonies.

Plate 2: 5 *Fusaria* colonies, 1 *Penicillium* colony, 2 *Curcularia* colonies, and 4 *Hormodendri*; the rest not identified.

Plate 3: 1 Acrothecium colony, 2 Hormodendri colonies, and 1 Monilia sitophila; the rest not identified.

Plate 4: 1 M. sitophila colony, 1 Cladosporium, 2 Hormodendri; the rest unidentified.

Plate 5: 7 Penicillia colonies, 2 Hormodendri, 2 Fusidia, and 1 M. sitophila.

The results confirm our opinion that it is impossible to arrive at definite conclusions with only one sample. This point of view agrees with an investigation conducted by Cadrecha and the author in 1939, exposing plates every two hours and comparing the results with different atmospheric conditions prevailing at the moment of each plate.

All the above is subject in great measure to the direction and intensity of air currents. Although no definite conclusions can be drawn, except that the samples must be taken many times during the day to reduce the margin of error, there is otherwise no reason to abandon the method used, since we are interested in obtaining enough samples in each season to determine the fungi variety dominant in each period.

In the photographs that will follow, we can appreciate the difference in each plate (Figs. 2, 3, and 4).

In the tropics, curves are altered in an arbitrary fashion, as can be observed in Figures 5 and 6, representing curves for several years.

Fall of the Spores

Spores for the most part affect a spheroid shape, although there are some that are elongated in one of their diameters. Shape, weight and volume are factors affecting the velocity of the spores, not to mention the influence of the air.

The fall of an almost spheric spore such as one of the Aspergilli is not like the fall of one of the Alternariae or Pestalotiae. In the first instance, the laws of the fall of objects apply to a greater extent. Theoretically, it could easily be calculated by means of Stokes law with its subsequent modified formulae. In the case of elongated spores, with the weight being more pronounced at one end than at the

other, other factors will have to be considered, which is simple enough. It is important to consider the hygroscopic factor, since the weight increases with the amount of humidity absorbed. Another factor to be considered, even though only theoretically, is the density of the different strata they will have to traverse.

The fall of a sphere is calculated by measuring its density, radius and fall rate. But each spore is covered by a thin film of humidity which will alter the calculations; were thus not taken into consideration, the applied formula would not yield accurate results.

Stokes law has been proven correct in the case of spheres having a diameter between 0.001 and 10 cm., but when these are smaller, the calculations are not accurate. Therefore, Millikan has calculated a new formula:

$$V = \frac{2}{9} \frac{p - 6}{u} g a^2 \left(1 + \frac{b}{p a}\right)^2$$

V: terminal velocity
p: density of the sphere
6: density of the medium
g: gravity acceleration

a: sphere's radius
u: viscosity of medium
p: pressure of gaseous medium
b: constant (6.25×10^{-4})
when p is measured in cm. of Hg.

If we compare the velocity of fall of a fungus spore and a bacteria, we could see how the latter falls more slowly because of its smaller size.

All this has been arrived at by a study of fall in well-known atmospheres and under experimental conditions, without air. This is not true in the case of atmospheric air, but we can say that in it, the bigger the organisms (spores), the greater the rate of fall. One must consider that in descending under conditions of relative dryness, the spore will be losing its own [sic], and will thus begin to fall less rapidly.

Spores belong to the world of "things that float and stick." In objects where their smallness allows them to float, gravity is not sufficient to pull them downward against

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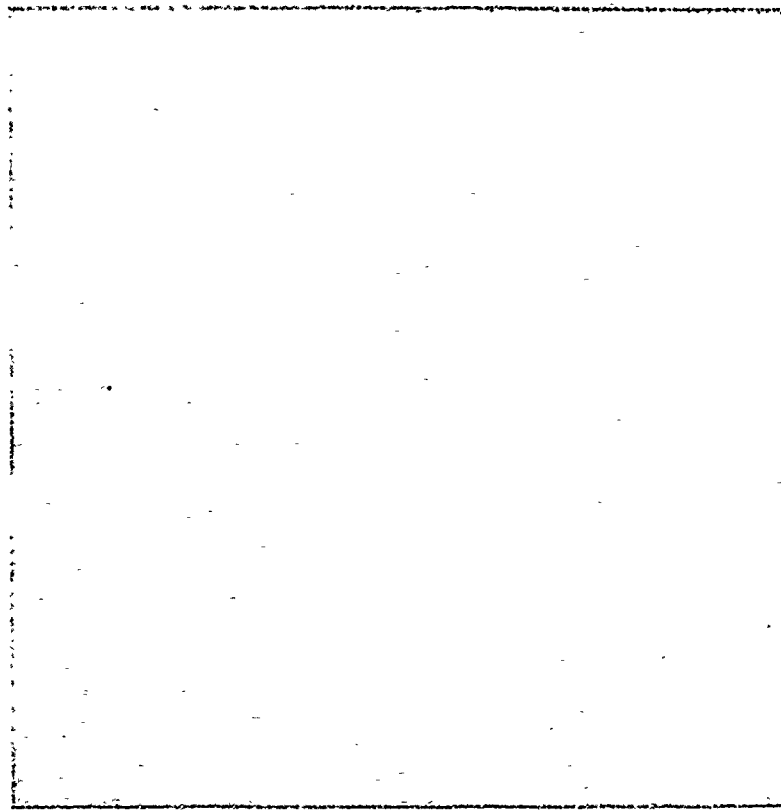


Fig. 2. Plate exposed for 6 minutes. Compare with subsequent ones exposed at the same time, but 3 meters apart.

air currents; and when they have been deposited, they stick and do not readily come off.

This condition of ingravity results from still another influence, the tendency to make them symmetrical, a characteristic shared by many of the organisms of that microscopic world. Thence its being known as the world of spheres. This is a characteristic of many spores and pollen grains. The best floaters, so to speak, are those between 17 and 58 microns.

Those exceeding such size are mainly "stickers", and although they do not float very well, they "stick" easily. The smallest ones, with their proportion between weight and surface, as is the case with the majority of spores, make

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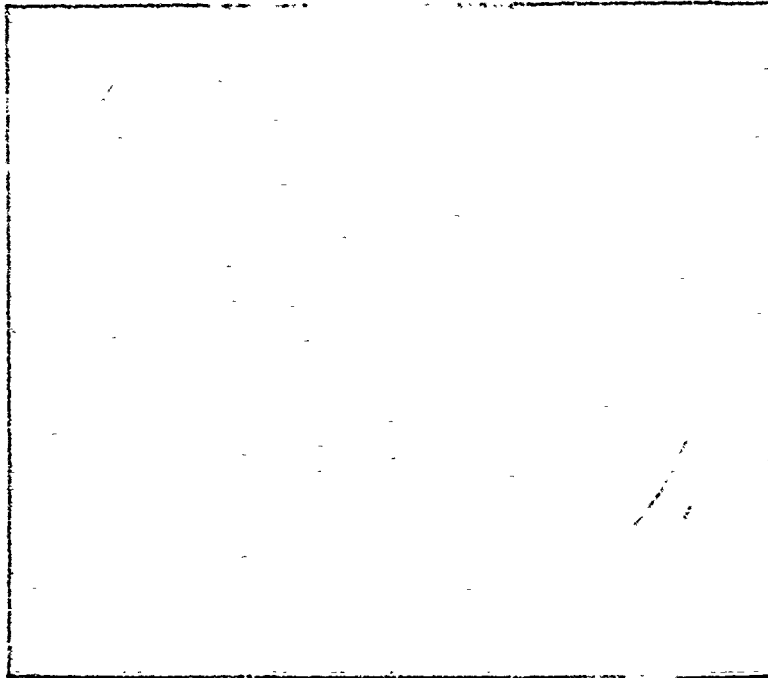


Fig. 3. Plate exposed at the same time as in Fig. 2, but 3 meters apart.

use of a take-off mechanism in order to free themselves and begin to float.

The mobility and transfer of spores is subject to a series of factors, the most important among which are: ascending and descending currents, atmospheric humidity, and the weight and volume of the spores. After getting off the conidiophores or similar elements, the spores are transported by the air and are impelled at different heights, and are gradually sedimented onto the earth, often very far from their point of origin. For this reason it is not always possible to infer that the fungi come from local sources.

Our main concern is, however, their composition and content in the air we breath. One way to find out the amount of spores we inhale daily is by their number per cubic yard of air. The air consumption per person is approximately 20 yards per day. Thus, for instance, if we had, let us say, 1,000 spores in a cubic yard, and the air consumption was 20 cubic yards daily, in 24 hours we would come in contact with 20,000 spores. This may not be so, since quantity and

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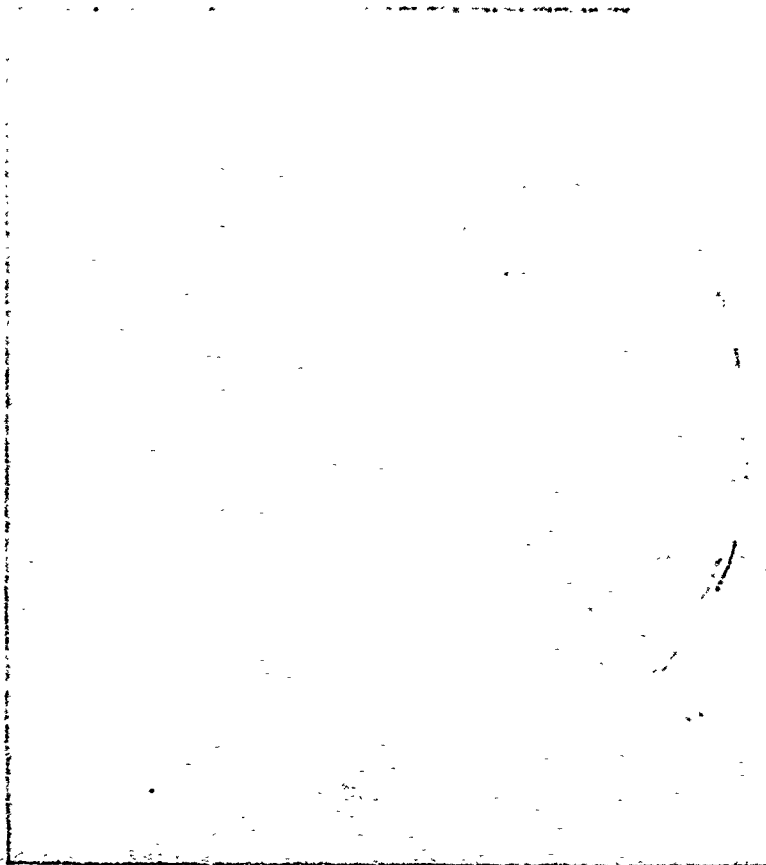


Fig. 4. Plate exposed 3 meters away from the previous ones. They were all opened and closed simultaneously to make them comparable.

composition of the air vary considerably. The figures are used to convey an approximate estimation. The quantities are possibly lesser.

This calculation is not difficult if the "small plate" per cm^2 method is used, raising it later to a cubic yard.

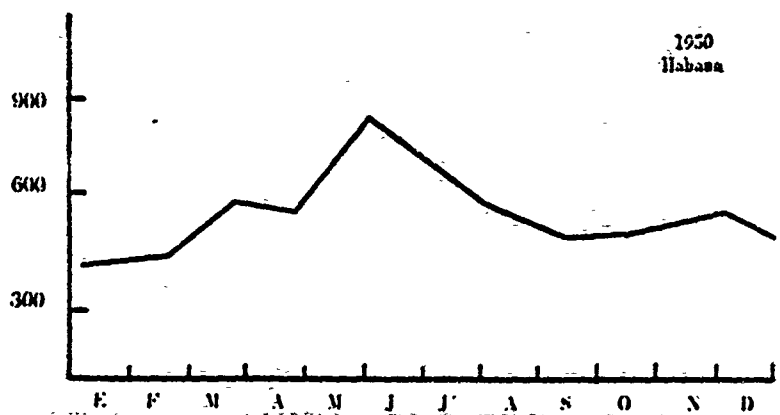
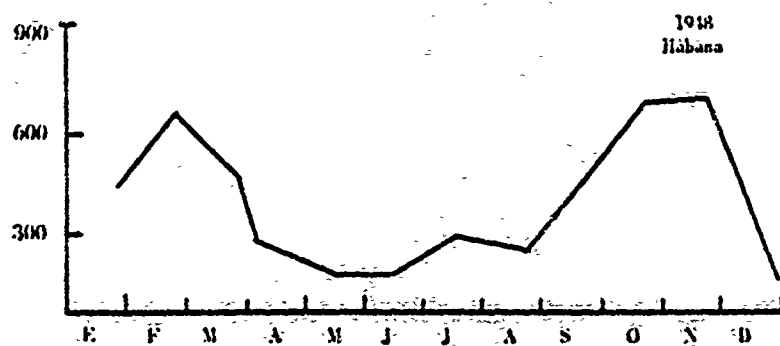
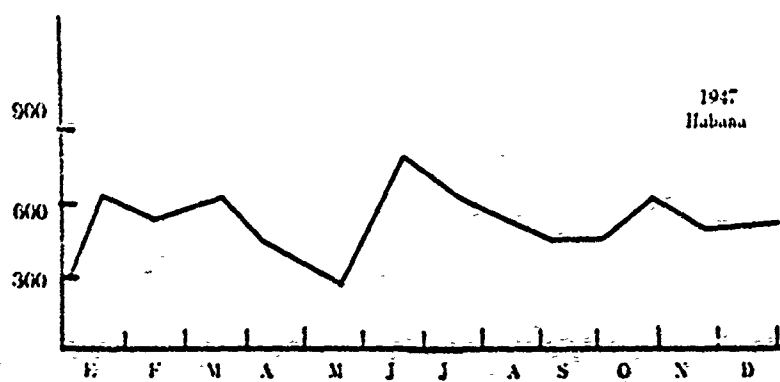


Fig. 5. Annual Monthly Average (Number of colonies per 12 minutes of daily exposure.)

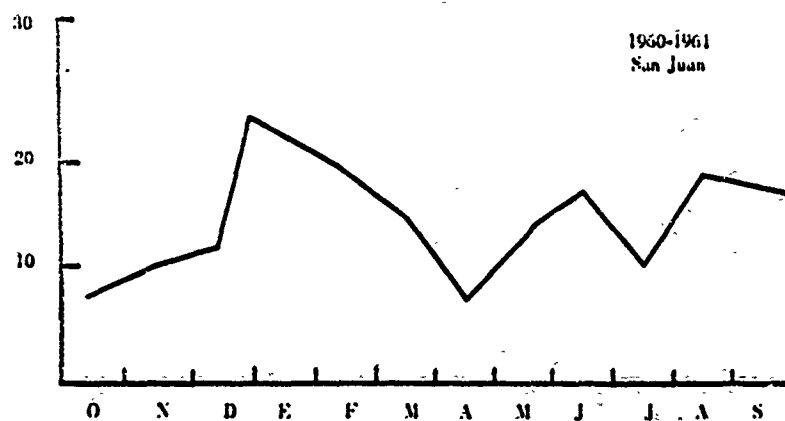


Fig. 6. Annual Monthly Average (Number of colonies per 12 minutes of daily exposure.)

Isolated Fungi (Puerto Rico)

<i>Blakeslea trispora</i>	<i>Gloesporium</i>
<i>Penicillium nigricans</i>	<i>Fusarium nivale</i>
<i>P. jantincellum</i>	<i>Fusarium</i> (Sección Arachnites)
<i>P. decumbens</i>	<i>Fusarium</i> sp.
<i>Penicillium</i> sp.	<i>Curcularia</i> sp.
<i>Fusidium carneolum</i>	<i>Chalaropsis thielavoides</i>
<i>Pestalotia</i> sp.	<i>Melanconium</i> sp.
<i>Periconia</i> sp.	<i>Cryptomella</i> sp.
<i>Nigrospora sphaerica</i>	<i>Hormodendrum</i>
<i>Lacellina</i> sp.	<i>Trichoderma viride</i>
<i>Verticillium candelabrum</i>	<i>Rhizopus arrizus</i>
<i>Aspergillus nidulans</i>	<i>R. nigricans</i>
<i>A. flavus</i>	<i>Monilia sitophila</i>
<i>A. tamarii</i>	<i>Cladosporium cladosporioides</i>
<i>A. glaucus</i>	<i>Spondylocladium</i> sp.
<i>A. luchuensis</i>	<i>Cephalosporium</i>
<i>A. parasiticus</i>	<i>Trichotecium</i>
<i>A. flavus-oryzae</i>	<i>Acrothecium</i> sp.
<i>A. niger</i>	<i>Mucor</i> sp.
<i>A. terreus</i>	<i>Actinomyces</i> sp.
<i>Aspergillus</i> sp.	<i>Stemphylium</i> sp.
<i>Helminthosporium sativum</i>	<i>Phoma</i>
<i>H. hadotrichoides</i>	<i>Acremonia</i>
<i>Helminthosporium</i> sp.	<i>Botrytis</i> sp.
<i>Neurospora crassa</i>	<i>Spicaria</i> sp.
<i>Alternaria tenuis</i>	<i>Chaetomium</i>

Incidence of Fungi in the Different Regions

This merits consideration because it allows a better knowledge of what takes place generally in great expanses. In the United States, for instance, the following occurs according to Prince:

Alternaria: Very frequent. In the north the amount is considerably reduced in winter but is abundant in summer. In the south, it is practically perennial. In the northeast, the quantity is relatively minor.

Hormodendrum: Abundant in the north with decrease in summer. Abundant in the south in autumn and spring with slight decrease in summer.

Helminthosporium and Spondilocladium: Abundant in the north in summer, and in the south in spring, autumn and summer.

Fusarium: More frequently found in the south.

In Cuba, predominance is as follows:

January to May: Hormodendrum and Monilia sitophila.

April to June: Hormodendrum, Mucor and Penicillium.

July to September: Rhizopus, Hormodendrum, Spondilocladium, Acrothecium, Penicillium, Fusarium and Monilia sitophila.

October to December: Spondilocladium, Acrothecium, Penicillium, Fusarium and Monilia sitophila.

In Puerto Rico:

Hormodendrum: Throughout the year, especially in winter.

M. sitophila: Abundant in winter for the reason given in relation to parasitism in sugar cane.

Acrothecium & Curvularia: Abundant from November to June, but also found all year around.

Fusarium: Predominates in the winter months, but may be present in other months without manifest predominance.

Alternaria: Generally appearing in summer on a few occasions.

Analysis of the preceding data shows that in the United States there is predominance of Alternaria and Hormodendrum; in Cuba, Hormodendrum, Aspergillus, Penicillium, Fusarium, Monilia sitophila, Sporidiobolus, Curvularia, Acrothecium and Rhizopus; and in Puerto Rico, Hormodendrum, Sporidiobolus, Helminthosporium, Curvularia, Fusarium, Monilia sitophila, Penicillium, Aspergillus, Fusidium, Trichoderma, Cephalosporium. Perhaps Rhizopus, Chaetomium and Nigrospora are more abundant in Cuba than in Puerto Rico, but Helminthosporium, Fusidium, and Pestalotia seem to predominate in the latter. It can be said, however, that there are not great differences between the two islands. (Drawings of some of these fungi are offered in the Appendix)

Detailed Study of Monilia Sitophila

The reason we have chosen to make a more detailed study of the fungus Monilia sitophila is that during part of the year its curve peaks very noticeably, leading us to think that its multiplication was due to particular conditions of the substratum at that time. Wishing to clarify this point, we decided on the following study:

1. Find out what parasitable material and what harvest predominated in winter months.
2. Expose plates in metropolitan areas, in the country, shore, and mountain, and in different parts of the city.

In the beginning of the study, we thought that the increase in spore count was conditioned to the parasitation of refuse from the sugar complex, since parasitation was high in refuse dumps.

We were not surprised at discovering almost pure cultures of Monilia sitophila in the fresh filter-press cakes deposited near sugar farms. Although this could be the cause of the dissemination and increase of Monilia, we suspected other sources of contamination. On that account, we studied the further uses made of sugar refuse, finding that it is very frequently used as a garden fertilizer in the metropolitan area. The next step was the study and comparison of fungi germination in them.

When fresh filter-press cake or "cachaza" was deposited, a great fermentation took place, resulting in heat release and harm to the herbs therein sown. At this stage, no fungi were to be found, but once cooled off, the soil samples showed a great profusion of Monilia sitophila germination, it being the only fungus present. Gardens were covered by large pink colonies typical of fungi as we can appreciate in the accompanying photographs. (Figs. 7 & 8)

The next step was a study of the means and method of transport used to get the filter press-cake to the gardens, which is usually done in small open carts that keep dropping fungi as they roll along.

On different occasions, we took samples of garden soils. We found that if the "cachaza" mixed in the soil was recent and had stopped fermenting, the monilia content was enormous; the opposite happened if the "cachaza" was old and dry.

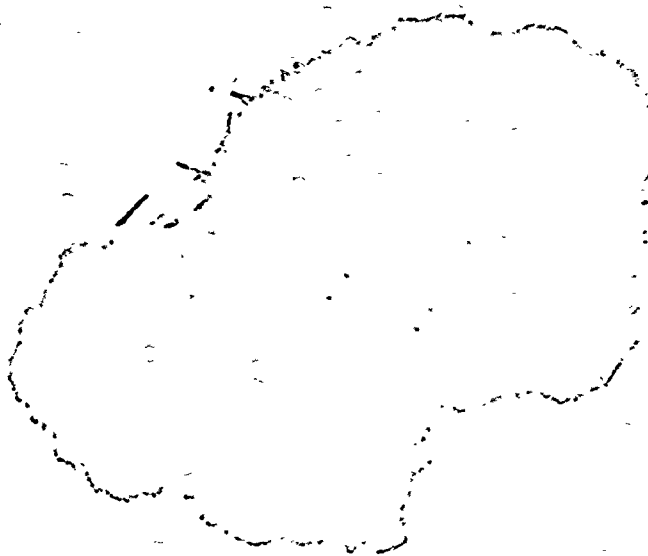


Fig. 7. M. sitophila colony in soil lump
Fertilized with "cachaza."

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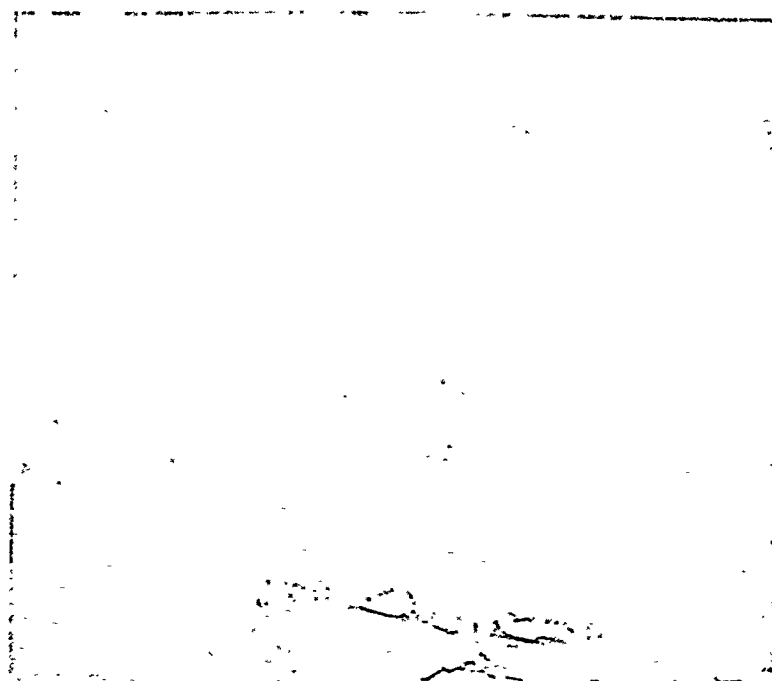


Fig. 8. Garden with M. sitophila parasitization. Typical fungus pink colonies can be observed.

Wishing to know the composition of such matter, we analyzed studies made on its chemical make-up by various investigators. At the same time, we used this refuse as cultivation ground utilizing "cachaza" from previous years through the kindness of Mrs. Maria del C.C. Fernandez, who was studying it for other experimental purposes.

Filter-press cake or "cachaza" has been comprehensively studied in Puerto Rico. Already by 1930, in a thesis submitted at Rutgers University by Dr. Juan A. Bonnet, we find the following interesting information: It is produced at a rate of 1.25 to 2.50 parts each 100 weight-parts of cane. Composition is as follows:

<u>Constituent</u>	<u>Percent</u>
Fibre	30-40
Soil	10-15
Wax	20-30
Albuminoids	10-15- 1.6 - 2.4N2
Ca3PO4	10-15 (5.4 - 8.1)CaO

Fibre is the insoluble element and is represented by cellulose, semi-cellulose and lignine. These are broken down in the soil by the action of bacteria and fungi. The relationship between the amount of nitrogen assimilated by the fungus and the air bacteria is around 31.1 percent.

The nitrogen from albuminoids that is not synthesized by the microorganisms would tend to accumulate as ammonia to change and become part of nitrites and nitrates by the action of microorganisms.

The group of fats and waxes merits consideration as it makes up 30 percent of the cake. Waxes and esters of high alcohols and fatty acids. The fats save the substances used by organisms in the absence of other sources of energy. They go through a slow breakdown in humid soils and hardly at all in dry soils. Waxes are even more resistant than fats; they act under aerobiosis conditions in their breakdown by bacteria and fungi.

Every year, Puerto Rican sugar mills produce enormous quantities at a rate of some 60 lbs. of cake per milled ton, as can be seen in the interesting research by Samuels and Landrau, published for the 9th Convention of the International Society of Sugar Cane Technologists held in India in 1954.

Assuming annual production to be 12 million tons of milled cane, the resulting "cachaza" will amount to 360,000 tons. This material is accumulated in stacks that cover many acres near the mills. Great quantities are used as fertilizer in the nearby cane fields, a other portion goes for equal purposes to the gardens, and the rest is left without use. Its use has proven satisfactory in pineapple fields and for different vegetables.

Furthermore, the cake contains a certain amount of lime added in the process of neutralizing and clarifying the juice; and sand and soil enter as contaminating agents.

One of its characteristics is the absorption of great amounts of water when it is dry. As we shall later see, this is important in fungi reproduction.

Another interesting data is that a ton of it with a 65 percent humidity would take up a surface of about 2 cubic yards.

Chart 7 shows the cachaza's chemical composition according to the latest studies (1954).

Chart 7

Chemical Composition of Cachaza

1) Constituyente	2) Muestras	3) Composición a base de peso seco	
		4) Promedio	5) Valores
	6) Número	7) Por ciento	7) Por ciento
8) Nitrógeno (N)	43	2.19	1.07 - 3.13
9) Fósforo (P ₂ O ₅)	43	2.77	1.34 - 6.30
10) Potasio (K ₂ O)	41	.44	.02 - 1.77
11) Calcio (CaO)	42	3.05	.98 - 6.24
12) Magnesio (MgO)	2	.49	.42 - .58
13) Manganeso (MnO ₂)	6	.17	.10 - .24
14) Hierro (Fe ₂ O ₃)	6	1.05	.26 - 4.71
15) Boro (B ₂ O ₃)	4	.01	- .02
16) Materia orgánica	1	39.5	-
17) Pérdida al incinerar	1	45.2	-
18) Sacarosa	4	3.0	2 - 4
19) Humedad (fresca)	18	61.0	56 - 69
20) Humedad (almacenada)	23	15.0	9 - 47
21) Peso-volumen	2	.375	9.372 - .378

[Legend]: 1) Constituent; 2) Samples; 3) Comparison Based on Dry Weight; 4) Average; 5) Values; 6) Number; 7) (Percentage); 8) Nitrogen (n); 9) Phosphorus; 10) Potash; 11) Calcium; 12) Magnesium; 13) Manganese; 14) Iron; 15) Boron; 16) Organic matter; 17) Incineration loss; 18) Saccharose; 19) Humidity (Fresh); 20) Humidity (Stored); 21) Weight-volume.

Again, regarding the study of contaminating material found in gardens, it can be said that the more humidity in the soil, the higher the amount of Monilia.

Making use of cake samples, we found that:

1. Sterile and dry "cachaza" does not parasitate well, but adding water turns it into a good culture medium, especially for Monilia sitophila.

2. Samples of old (2 years) "cachaza" showed different fungi, but without predominance of Monilia. It seemed to require fresh cake for the fungus to develop intensely and to predominate.

3. Penicillium was dominant in acidulated "cachaza" samples.

4. In sterile and dry "cachaza," neight Aspergillus parasiticus nor Fusidium, Cladosporium, or Pestalotia, and not even Monilia satophila developed well. All the foregoing seems to prove that maximum multiplication of Monilia is related to some substance present in fresh filter-press cake.

5. On adding water to dry cake, we found that Monilia immediately predominated over the other fungi, which became then hardly noticeable.

The abundance of Monilia in the San Juan and Ponce air near the sugar mills did not correspond with any marked increase in the island's central region, showing that it exists in greater amount where there is abundance of contaminating material.

Culture Media

There is great variety of culture media to be used in the preparation of our extracts and in the isolation and identification of the colonies. Based on their simplicity, we could classify them as follows:

1. Isolation and Identification:

Sabouraud	Petri dishes
Agar glucose potato	Id.
Mehrlich	Id.

The main difference lies in the rapidity of development of the colonies, the fastest one being agar glucose potato, Mehrlich was the slowest. In places where there is abundance of fungi, it is better to use the "slower" media.

2. Colony culture:

Malted broth
Broth with added juices and vegetable substances.

Synthetic broths -- Czapek, Center, etc., the best being the Center media.

These broths should only be used to get the colony to grow in known, antigenic medium and later as a diluent. The reason for synthetic media is the need to eliminate the medium's antigenic factor.

Preparation of Antigenic Material

Let me say that colonies can be used directly from the dishes once the appropriate liquid has been added. Naturally, this has its limitations. The same is true of the use of spores as allergen, rejecting the middle and rest of the micelle. This is only possible with certain fungi that release their spores easily. I do not believe it necessary here to pause in detail over the different methods, and will deal only briefly with this aspect.

We have used a method based on the release of the spores directly from the solid medium and gathering them in graduated centrifugal tubes (Fig. 9). This permits the addition of known quantities of the extracting liquid, and, after having allowed a certain time to pass between its agitation in a shaker and its refrigeration, final passage through Seitz filters.

Likewise, we found it practical to prepare extracts making use of the union of the spores and the micelle, finely pulverized and placed in centrifugal concave-bottom flasks, then shaken for many hours in a shaker after having added chrystal pearls (Fig. 10). This method yields antigenic extracts richer than those obtained in the conventional fashion without breakdown or agitation. Besides, less extracting liquid is lost and manipulation is faster and more accurate.

Selection of Method

The preference over any one of the following methods, we believe, depends on the particular characteristics of each fungus. Thus we have:

1. Fungi releasing a certain considerable amount of their antigen in the extracting liquid, although mixed with products of their metabolism and growth.

2. Fungi whose allergenic capacity centers mainly on their spores.

3. Fungi whose extracts must be enriched, making use of both fractions.

The first method utilizes the synthetic media and may be purified later if so desired. The second one uses spore culture, and the third one is a mixed method. The most widely used method is, in any case, still the one of extracts derived from micelle and from the spores, and deprived of culture medium.

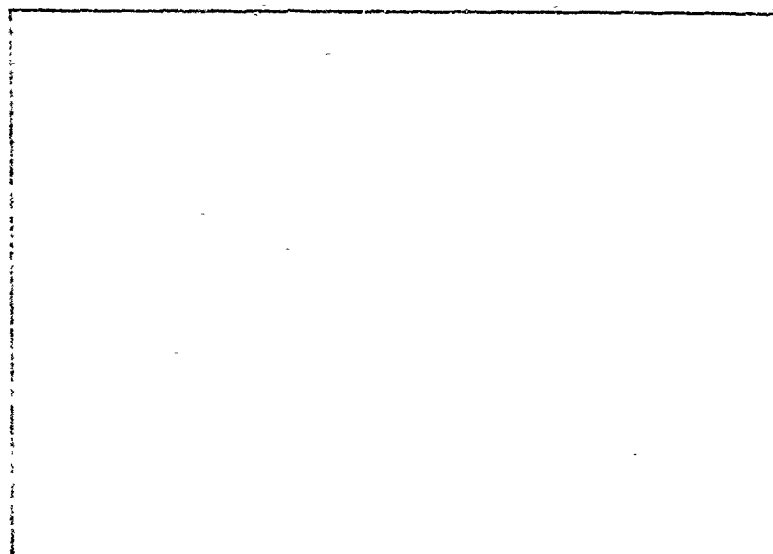


Fig. 9. Fungus spores taken directly from culture.



Fig. 10. Pulverized micelle and spores in extractive stage.

There are many opinions as to the best way to prepare antigenic material; therefore, investigators have used many methods. Some have made exclusive use of the extract made from the dry and pulverized film after cultivation in an appropriate liquid. Others, meanwhile, believe the best method to be the use of a synthetic liquid medium where, during the reproduction of the fungus, products of its metabolism are derived, and where among the latter the antigens will be present.

There are multiple variations of the two principal methods. We think the first one is the most convenient and practical, since on the patient we are interested in using material extracted directly from the spore and the micelle without mixture of metabolic products. There is

also a series of minor factors such as the lack of complete development of the fungus in synthetic media. Although some fungi reproduce well enough in them, there are others wherein the micelle develops but where asexual reproduction through conidia is poor instead. This criticism may not apply in case of fungi of the Candida type, where there is an obvious advantage in the synthetic medium.

Throughout the years, we have used the following methods:

1. Extracts of pulverized film in the saline buffer solution.
2. Extracts of pulverized film in saline glycerine solution.
3. Spore extracts in buffer or in saline solution.
4. Pooled extracts from different species.
5. Film extracts treated with alcohol.
6. Film extracts without alcohol.
7. Extracts precipitated with acetone.
8. Extracts derived from synthetic culture medium with adjusted pH.
9. Film extracts obtained through dialyzing membranes.

Of course, each one of these methods has its own natural advantages, depending generally on the type of fungus.

Were we to choose among all those listed so far, we believe we would do it in this way:

1. Colonies obtained after complete development -- in buffer or glycerine solution, previous death by alcohol, dissection or pulverization, and placed in automatic shaker during 48 hours for a better extraction and homogenization. A malted broth.

2. Fungi with unicellular tendency, and other fungi chosen from synthetic media, which shall be used as mother-extract after adaptation of the pH. Occasionally, precipitates shall be prepared in an attempt to purify them. A synthetic medium.

3. Conidia extracts in buffer or glycerine. We think this is the most rational method, although it does not permit one to obtain great quantities of extract.

As can be expected, there are differences in the potency of the resulting extracts depending on the conditions peculiar to the stock. Sometimes we have to extract from the same fungus, yet different stocks whose asexual reproduction is rich in one and poor in the other. This in itself explains the variability of the potency. Hence we must always prefer extracts made with well-developed fungi. In the particular case of Aspergillus, such difference in distinct cultures. Aspergillus should always be given to the one producing the most spores.

Non-Synthetic Medium

In the case of extracts prepared directly from films obtained in non-synthetic media, the following steps are necessary:

1. Sample take in petri dish.
2. Identification of colony and spores, previous sowing in tube.
3. Sowing in non-synthetic, liquid medium.
4. Alcohol treatment of the colony when the conidia have reached their maximum development.
5. Drying in glass bell under Cl_2CA .
6. Pulverization
7. Weighing
8. Separation of a sample for provocation tests, suspending the rest in extracting liquid in proportion of 1:20 in 35 ml. blung-botton centrifugal with glass pearls.
9. Constant agitation in automatic shaker three hours in the morning and afternoon for two days. Keep in refrigerator whennot being shaken.
10. Filtration -- Seitz -- Can in sterile flask labeling in P.N.U. or in weight-volume as desired.

11. Sterility tests.

Spore Extract

When wishing to obtain extracts free from micelle, which is only possible with certain fungi, extracting liquid shall be added to several culture tubes sown to the same fungus, then shaken so the conidia will be released. After extraction similar to the above mentioned method, conidia are counted by means of a counting chamber. Likewise, conidia may be separated for conjunctive tests, getting them from the bottom of a tube after recovery of the liquids used in suspension. In this case, saline serum is preferable to a buffer solution. Weak spores can best be kept by previous and direct suspension of them in 95 percent alcohol which allows quicker separation. Alcohol can then be eliminated by letting the spores dry; these must then be kept in well-sealed tubes in a refrigerator.

For simplicity's sake, I have only described the two easier techniques in practice in the separation of antigenic material. The difference in antigenic potency between these two and other methods is of minor importance if the material to be extracted is properly selected, is allowed sufficient time, and is carefully counted.

Synthetic Medium

A synthetic medium can be used, and the colony pulverized in a blender. In this case the procedure is different. It is advantageous to apply it to certain fungi because no antigenic material is lost, although we are adding metabolistic products. The preference of one fungus over another depends to a great extent on the allergist and on the particular fungus.

There are times when certain inert substances must be added to make the fungus float and to obtain aerial forms and better sporulation, or perhaps use only the synthetic liquid without micelle.

A second part of this investigation that we thought important is this:

1. Study of the antigenic power of each fungus and its crossed reactions with others of like family.

2. Study of human sensitivity by the use of tests and serum studies in allergic patients.

In order to complement this work, we have started conducting Gel Diffusion tests on several fungi; Alternaria, Curvularia, Hormodendrum, etc., and also to make hemoagglutination tests that will eventually result in another published work.

Regarding the allergenic role of the majority of the fungi found in this study, we are able to provide information concerning their allergenic power. These studies were previously made in Cuba with its percentages included in clinical conclusions, and checked in the following fashion:

1. Positive tests by scratch and intradermoreaction.
2. Positive passive transfers.
3. Positive conjunctive and provocation tests.
4. Satisfactory treatments with the extracts.

Allergy Reactions in Human Beings

Air-floating fungi produce several allergenic respiratory manifestations translated into either a nasalsinus allergy or an intense asthmatic condition. In the belief that that it will be of interest, I shall offer briefly a part of the clinico-allergic aspect of such sensitivity, basing my concepts exclusively on the revision of clinical histories of patient studied in the last 15 years in order to point out the characteristics and diagnostic criteria of such allergies.

Although in 20 percent of the patients we had some positive intradermoreaction tests, this does not mean that all of those presented clinical symptoms, or that they were really sensitized to fungi. Proof is in the fact that there was negativity in nasal and conjunctive tests, and also in the search for circulating anti-bodies. In 10 percent, however, the tests are very definite, by any method used, and mucose tests and immunological studies permit confirmation of the diagnosis. I will dwell on this in an attempt to eliminate all cause for error in appreciation. PK tests permit confirmation of the diagnosis in a great number of cases.

Still, there are cases with very direct skin tests that do react to the conjunctive test, to spores in the nose or conjunctive, that we can therefore consider as really allergic.

In statistics compiled from our Cuban patients we found that 48 percent of fungi sensitivity is manifested as an asthma-ronitis, or the inverse, according to which symptom predominates.

Ronitis appears by itself in 18 percent of the cases; conjunctivitis in 28 percent, and asthma by itself in 34 percent. On occasions, fungi have helped produce an atopic dermatitis (4 percent) or a migraine (2 percent).

Analysis of the symptoms will reveal that the infection, whether it is nasal or oculonasal, will predominate, and will appear together with ronitis, the latter sometimes associated to sinus, to polyposis, or to an asthma present in one half of the cases under study.

Clinically speaking, fungi allergy has its own characteristics that I want to enumerate:

1. Aggravation in summer that occurs mainly in pure cases, although the symptoms may be present all through the year, or at the beginning, or in the winter, particularly when the fungi associate with other allergens.
2. The patients suffer more in the country and especially in humid places, cellars, sugar mills, breweries, vegetable and leather warehouses, lumber mills or near parasitical harvests, etc.
3. They tend to show symptoms on coming in contact with contaminated materials or plants, sometimes with pillows, and with shoes and other items covered with fungi colonies.
4. They may also show symptoms when drinking beer, eating fresh bread or foods containing yeast. But this is not always the case.
5. There is frequency of incidence among sugar mill workers and among farmers, gardeners, botanists. Yet, a considerable proportion exists in housewives and even in school children, which proves that any allergic individual may acquire that type of sensitivity.

6. Aggravation occurs on windy days, and frequently the smell of freshly watered earth bothers them.

7. Daytime aggravation, but without exclusion of nighttime symptoms, especially if they sleep in a room with high mycotic or allergenic content.

8. No relationship between sensitivity to air fungi and sensitivity to penicillin even though they may coincide. We have seen many instances of positive tests to Penicillium notatum in subjects not sensitive to penicillin.

9. Thirty-four percent of those allergic to fungi show symptoms in the summer, although only 28 percent do so in winter (generally from allergenic association or from climatic conditions). Twelve percent show the symptoms all year; while 26 percent show them only when in contact with the mycotic allergen.

If we make a detailed analysis of those ill from sensitivity to fungi, we shall see that sensitivity was the sole cause in only 6 percent. In 92% it was associated with some other cause, usually inhalants in the house, and in only 2 percent was there suspicion that a different allergen was present, although it could not be proved.

This makes us consider the need to go deeply into the allergenic study of a patient when it is found or presumed that the patient is sensitized to fungi. It is also convenient sometimes to consider that a positive test of the allergen is due to contamination of the material used to manufacture the extract or to allergens added to it. But we must and will bypass several significant considerations in order not to stray from the main theme.

Symptoms may appear at any age: we found them as early as in three years olds and as late as 55, with cases of symptoms after 70 years of age. We find that in 28 percent of the cases they appear between the ages of 1 and 10 years.

From 10 to 28 years of age	in 24 percent of cases
From 20 to 30	" " " " 26 " " "
From 30 to 40	" " " " 3 " " "
From 40 to 50	" " " " 6 " " "
From 50 to 60	" " " " 2 " " "

In women we find it in 38 percent of incidence, while in men 68 percent, possibly on account of the latter coming

more often in contact with parasited material. The average age for symptom appearance was around 20.

The origin of atmospheric fungi always leads to the find of parasitation in the different botanical products of the soil, harvests or dead plants on which they live saprophytically once dry, and also the refuse of industry.

As proof of this we will list a few atmospheric fungi, indicating at the same time on which plant or fruit they thrive parasitically.

Alternaria, on potatoes.

Hormogonium & Cladosporium, on tomato, peach, malt or stored tobacco.

Helminthosporium, on grasses and barley.

Monilia, on different fruits. Filter-press cake from sugar mills is highly parasited by Monilia sitophila.

Rhizopus, on sweet potatoes and other vegetables.

Fusarium, on cereals, cabbage, tomatoes and potatoes.

Mucor, on apples and pears.

Roya, on cereals, trees and fruits.

Blights, on grasses.

Schizophillum, on tree bark and on pieces of lumber.

It is not necessary to live near the harvested products or fruits above to find a good amount of fungi in the air, for it has been proven that fungi can be found at an altitude of 11,000 feet, and even in the Arctic, where they have been located during flights. They also travel great distances and on occasion in such large quantities that their passage has been noticed in different places for several days. They are found generally in quantities that vary from 100 to several thousands spores per cubic foot of air, the number depending on many factors and the season in which the study is made. In Cuba, where we made daily counts during the whole year, an increase takes place in the summer months, although not in fixed amount, and with fluctuations as to the type of fungi to be found, permitting us to make a more precise judgment of their allergenic role. Not all fungi have the same allergenic capacity, nor do all the shoots in the same fungus, which points out the need for continued improvement of our diagnostic and therapeutic material by adapting it to the findings in the air.

Besides fungi of the environment and those that are parasites of a thing or of a particular area, and whose collection and study will be now discussed, we must also consider, even though briefly, those of industrial and commercial

use, some examples of which I will just mention. Among industrial ferments, for instance, we have the different yeasts used in the making of beer, bread and certain cheeses. In other words, the Sacchromyces cerevisiae, elliposoides, Penicillium camemberti and roqueforti, and Mucor casei. Among foods, we have the mushrooms such as Psalliota campestris, certain Asiatic sauces, and the so-called Jew's ear, whose scientific name is Helvella edulis. Medical use of some fungi is represented by antibiotics such as Penicillium notatum and chrysogenum, and Streptomyces griseus and takadiastase which is but a diastase obtained from Aspergillus oryzae. Although several positive tests may be established on a patient, this may be caused by a group reaction of similar fungi, while at other times it means different sensitizations being definitely established by means of passive tests and search of the dominant antigen.

Among the fungi most frequently appearing in the air, we have the following: Hormodendrum, Acrothecium, Chaetomium, Mucor, Rhizopus, Fusarium, Nigrospora, Schizophyllum, Torula, Morilla, Helminthosporium, different Penicilliae, Chaleropsis, Aspergillus, Spicaria, Phoma, Curvularia, Sporosilocladium, Trichoderma, etc.

The diagnosis of this type of allergy is arrived at with previous knowledge of the air and zone where the patient resides, by use of tests either scarification or passive, the latter only in those cases where strictly necessary. At other times, it may be a conjunctive or nasal test, using a small quantity of spores directly applied with a special atomizer, or better yet, a toothpick; and it is of great value, permitting us to know the mucosa's direct response.

The treatment is effected through the fungus' diluted extract or a combination when the causatives are various, either combined with other allergens or not if their allergenicity were established. Sometimes in order better to judge a vaccine tolerance, we must do it separately. Whenever possible, hyposensitization must at the same time be combined with a diminution of mycotic contact. The time of the treatment, the interval between injections and the different doses will depend on the case and the patient's degree of sensitivity as it would be mistaken to follow a universal rule. The results are very encouraging after a few weeks of treatment. This must continue for months, with rest periods in the case of seasonal manifestations. It is then advisable to renew treatment 1 or 2 months before the recurrence of the symptoms.

The "repository" method may also be used, that is the slow absorption of a large amount of the allergen, emulsified in a special oily medium. Special equipment which will yield extremely fine and stable emulsions is used in its preparation. We think its use is decidedly advantageous in certain cases of inhalant allergies, especially pollen and fungi, as it would spare the patient many injections.

A later project could be the clinical study of patients sensitized to these elements, and, at the same time, the animal immunological study of the different fungi found. Part of it has been commenced by sensitization of laboratory animals and by obtaining immunoserums for precipitation tests and Gel Diffusion.

Summary

While pollen is the main seasonal causative agent of respiratory allergies in the Temperate Zone, in the Tropics this role belongs to the fungi in the air. This is because while abnormal quantities of pollen are released in the Temperate Zones during certain seasons of the year, this does not occur in the Tropics.

This Bulletin reports studies of the allergenic action of fungi in the Tropics, with particular reference to Cuba and Puerto Rico, where the author carried on his investigations, and the results of many clinical tests in Cuba. The causes of the variations in the fungi content of the air, both as to quantity and quality, are thoroughly explored, and a comparative study is made of the mycological flora of Cuba and Puerto Rico. The relative climatic stability of both countries accounts for high fungi content in their air, and the similarity of their mycological flora.

Since such climatic factors as direction and speed of the wind, rainfall, temperature, barometric pressure, and so on have a direct influence on the mycological flora of each zone, an extensive and detailed study of the climate was made.

In discussing the methods of exposing the petri dishes, it is recommended that the exposures be made at periodic intervals, at different times, in different locations, and under a variety of atmospheric conditions, which will assure more representative samples of the fungi in the air.

Although fungi spores of all classes are often present in the air, this Bulletin is especially concerned with

the genera of the Fungi Imperfecti because of their abundance and permanence at all times and under all conditions. These fungi are asexually reproduced by means of conidia, and their classification is based on the type of fruiting body and the size, shape, and color of the spores.

Because of the increase of Monilia sitophila during certain times of the year, a detailed study was made of the species. The results revealed a direct relationship between its presence in the proximity of sugar mills and the abundance of fresh filter-press cake or "cachaza" on which it grows, and its secondary use as fertilizer.

This Bulletin then outlines the methods of culture, preparation of antigenic material, and techniques employed, and discusses the advantages and disadvantages of each.

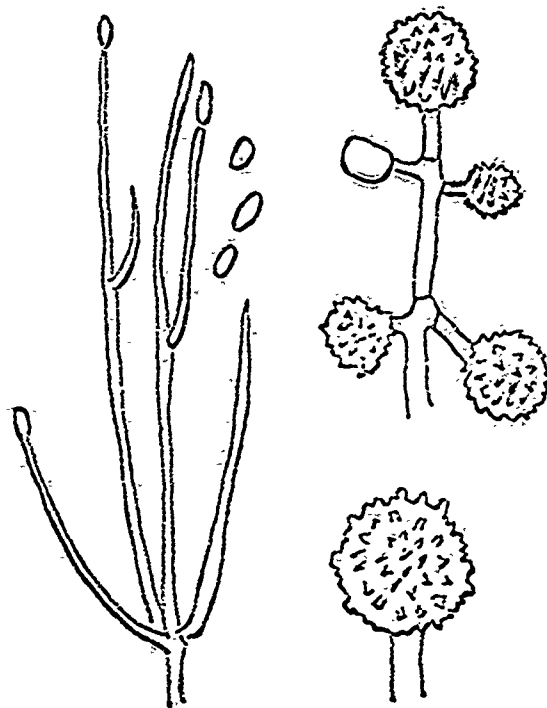
Separate lists are given of the most common species of fungi found in the Caribbean area, as contrasted with those typical of the United States, allergies, their primary symptoms, their peculiar characteristics, and statistics of incidence as to age and sex and other factors. Diagnosis and treatment are finally discussed from information based on the author's own experience.

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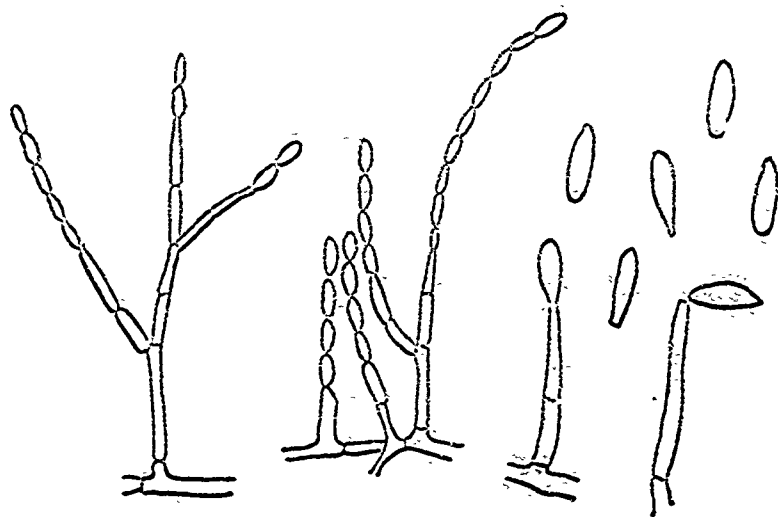
APPENDIX

Some of the Atmospheric Fungi of Puerto Rico¹

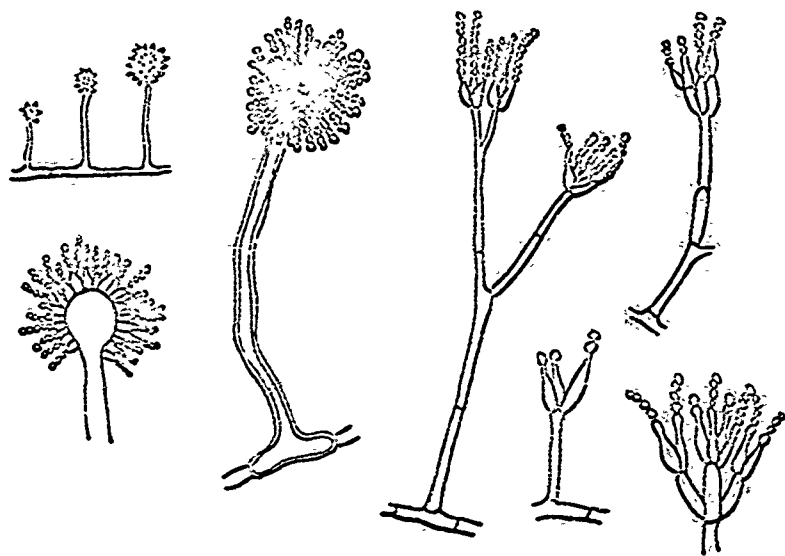


Sepedonium

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Fusidium

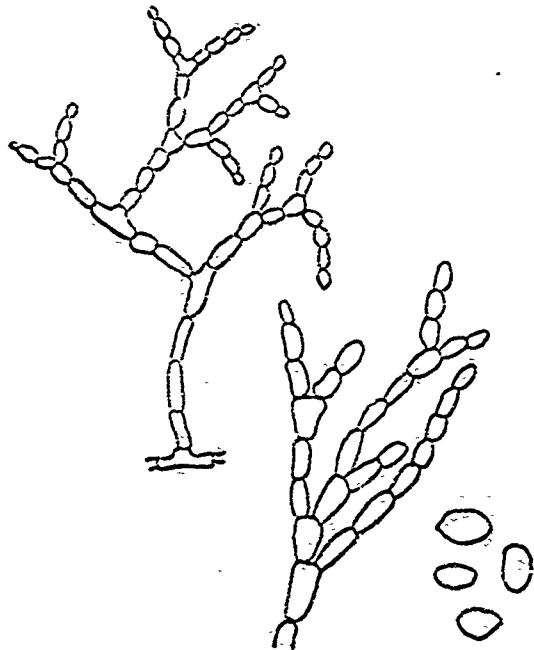


Aspergillus

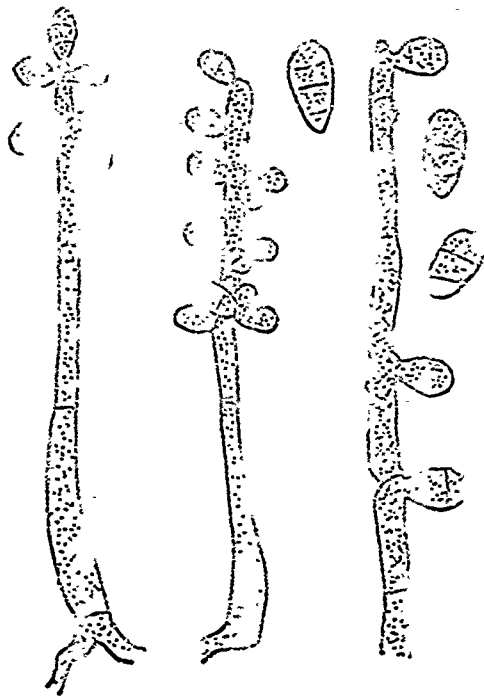
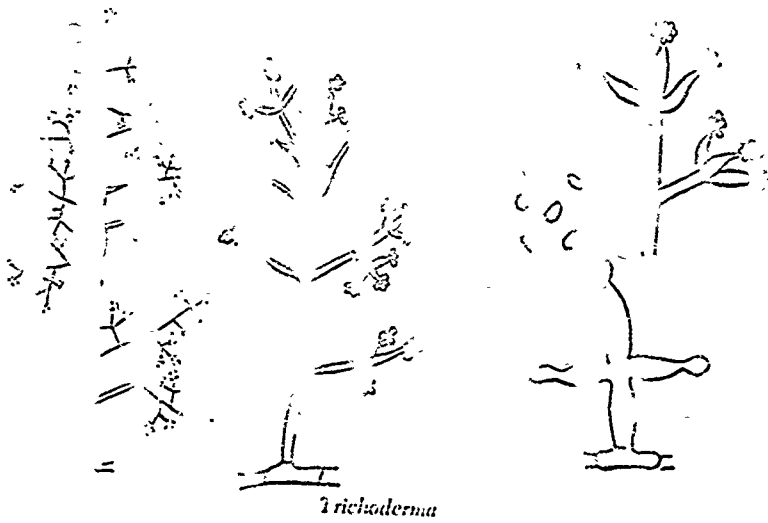
Penicillium



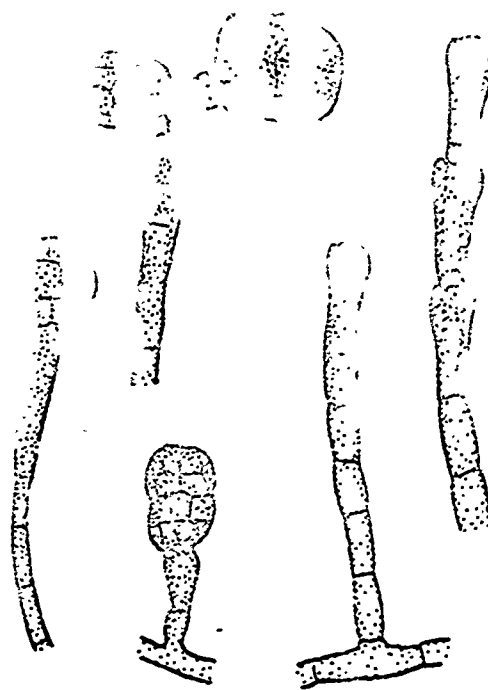
Botrytis



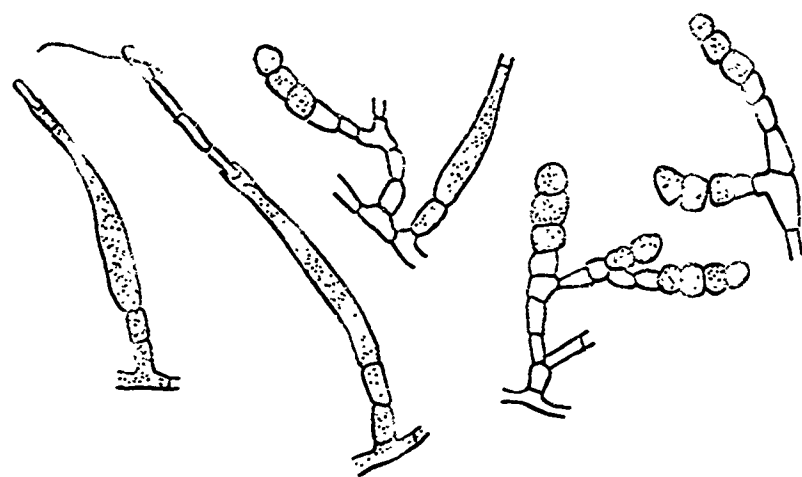
Monilia



Trichoderma



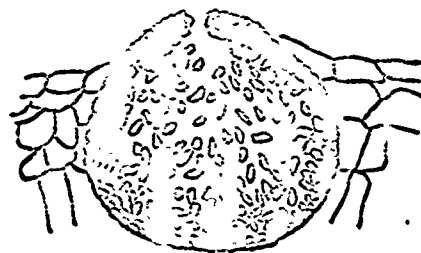
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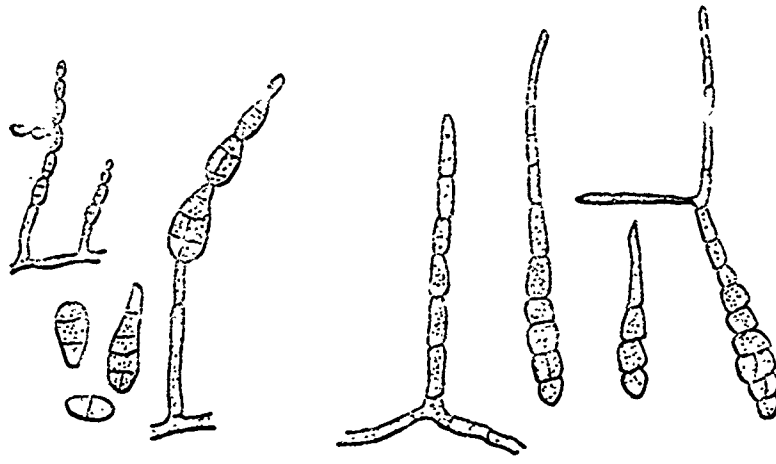
Thielaviopsis



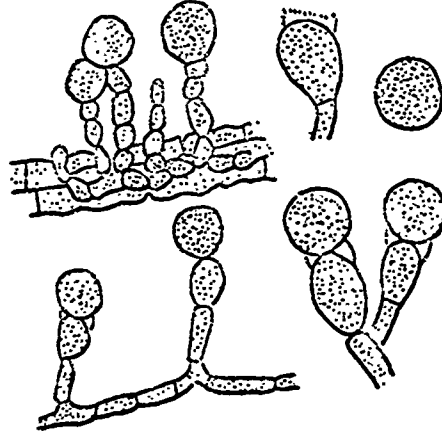
Hornodendrum



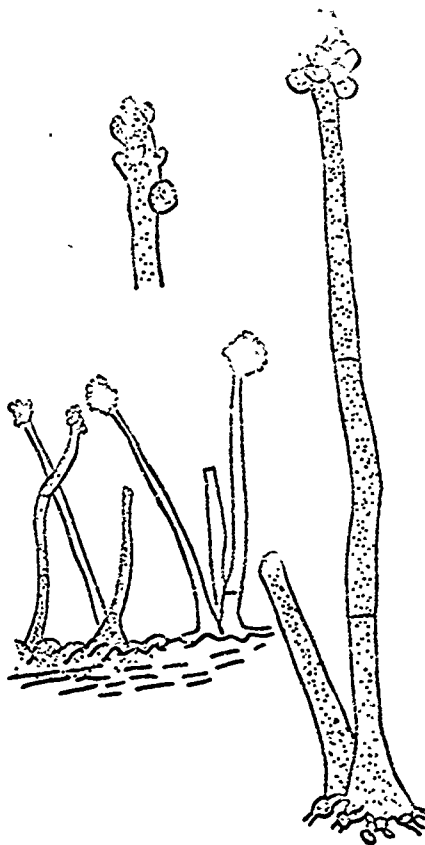
Phoma



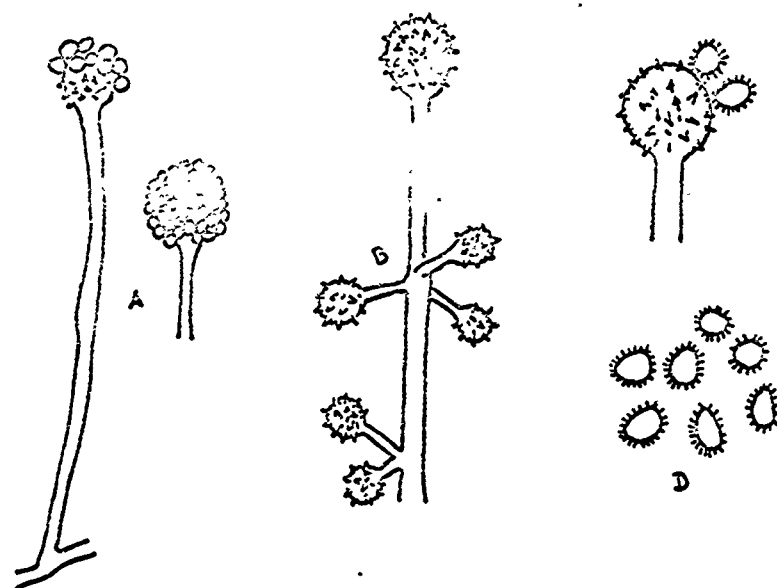
Alternaria



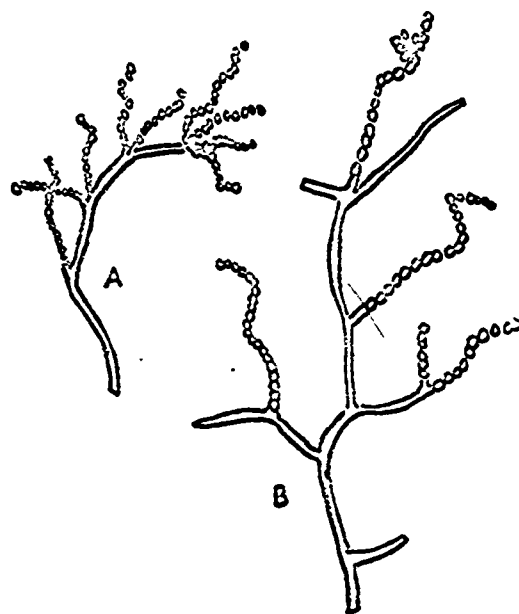
Nigrospora



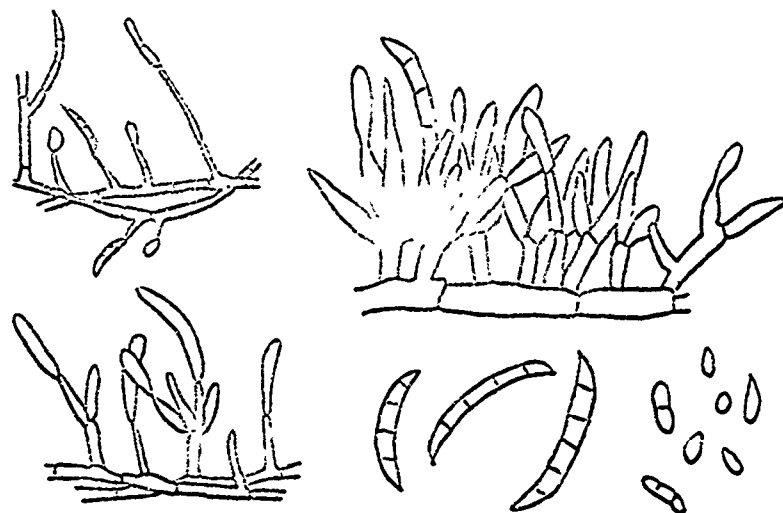
Periconia



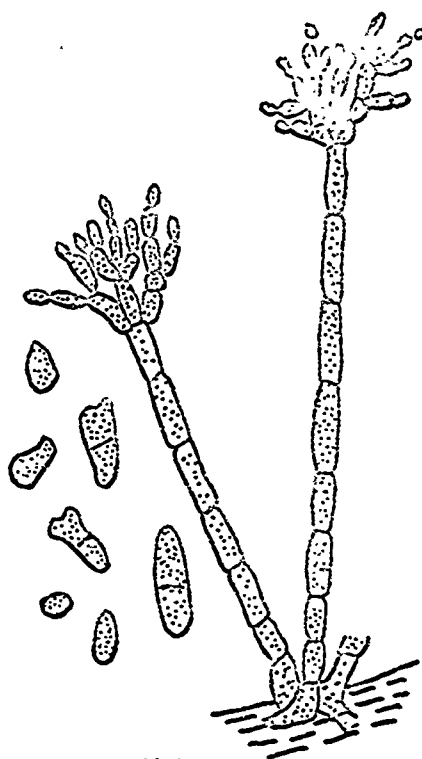
Cunninghamella



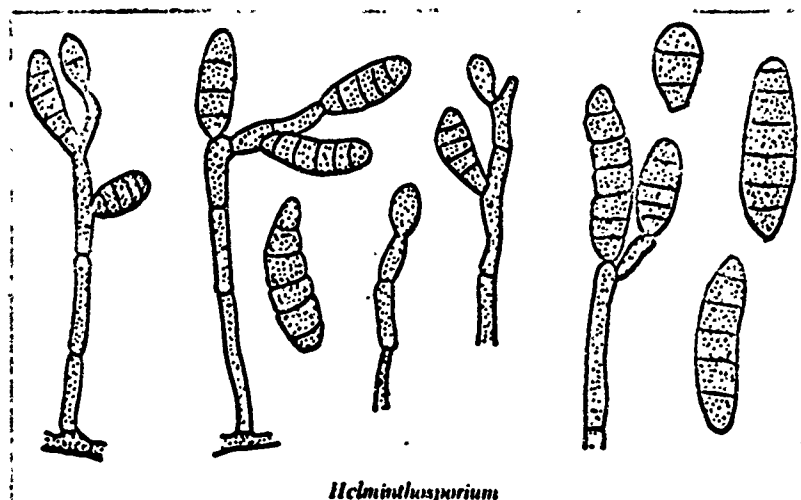
Streptomyces



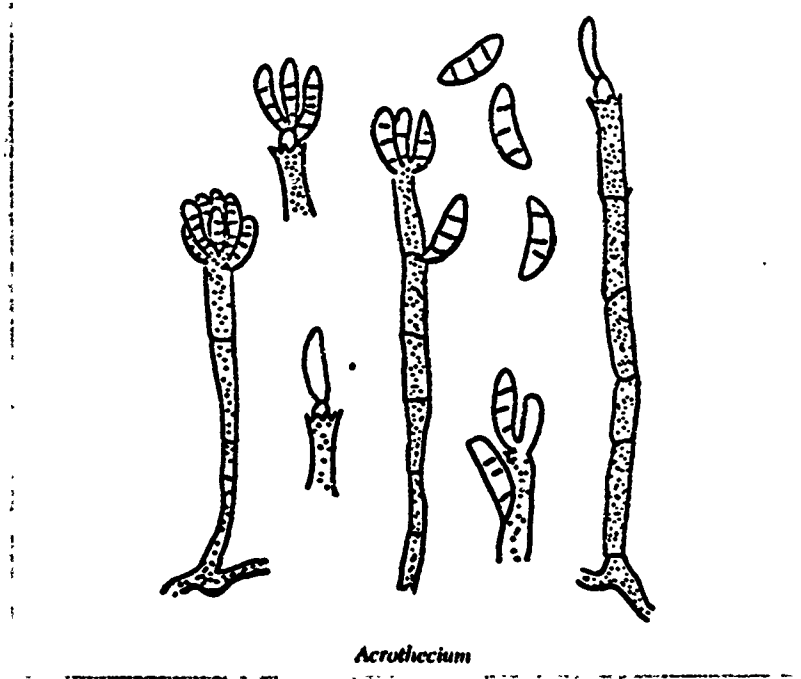
Fusarium



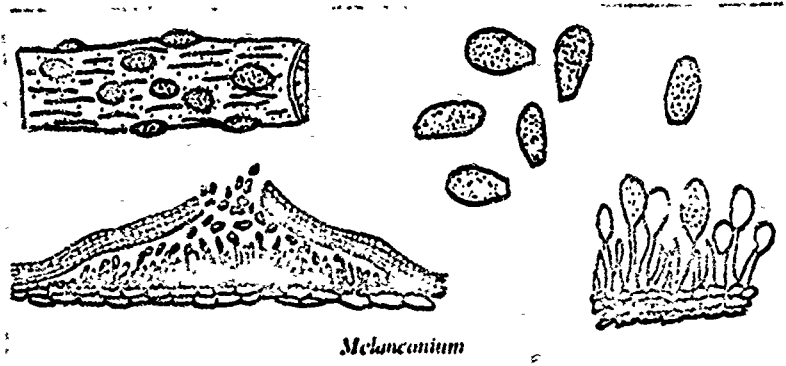
Cladosporium



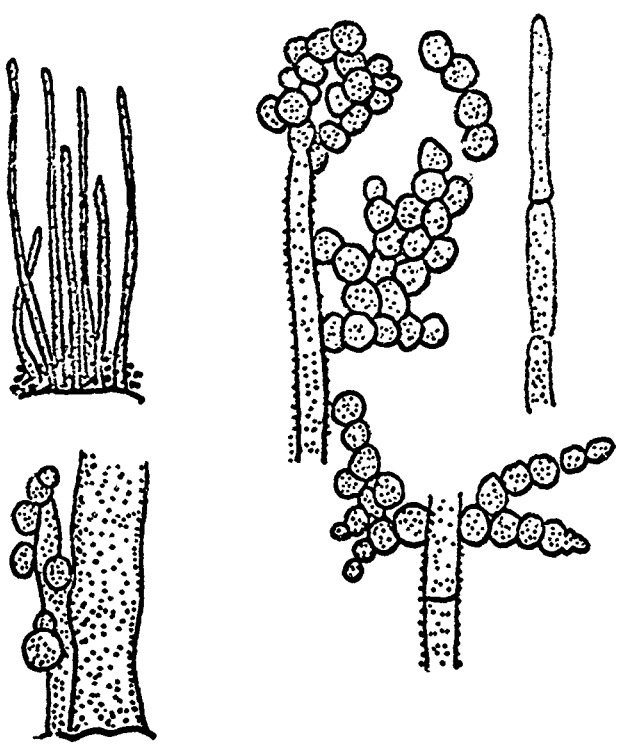
Helminthosporium



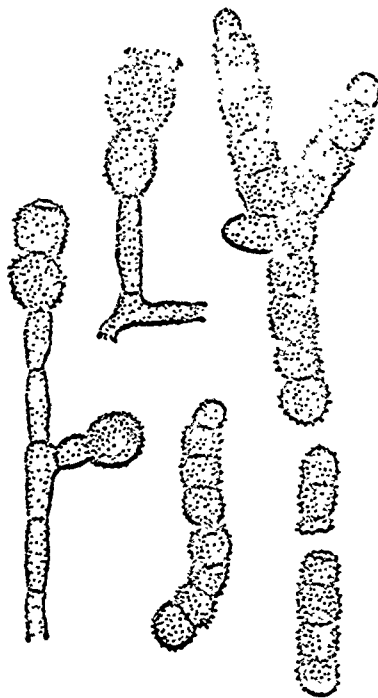
Acrothecium



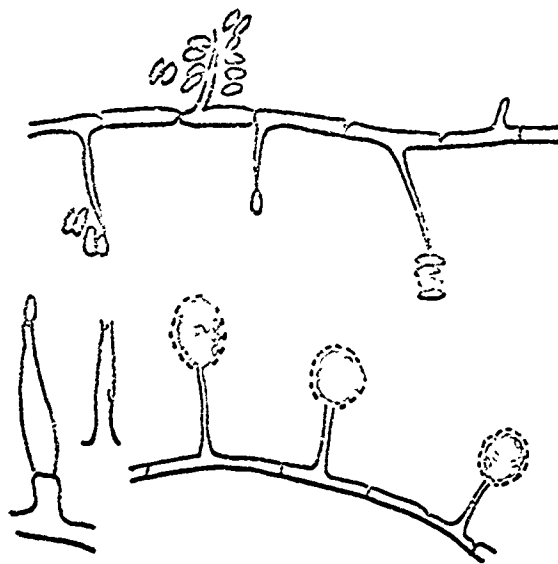
Melanconium



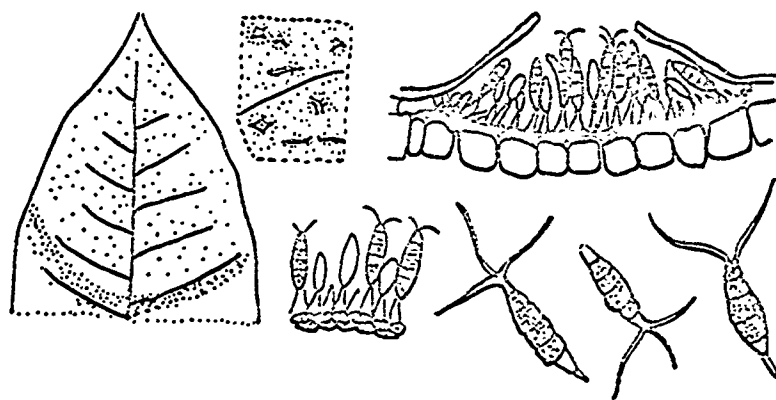
Lacellina



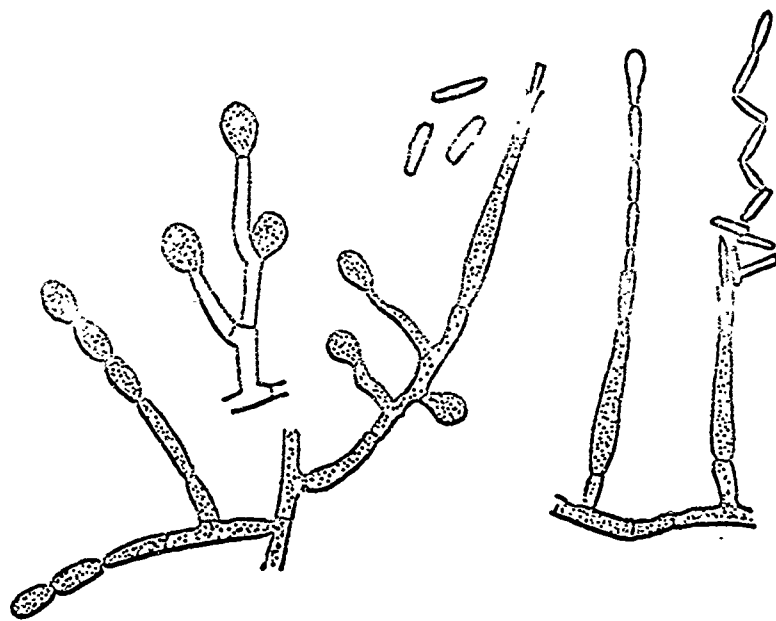
Torula



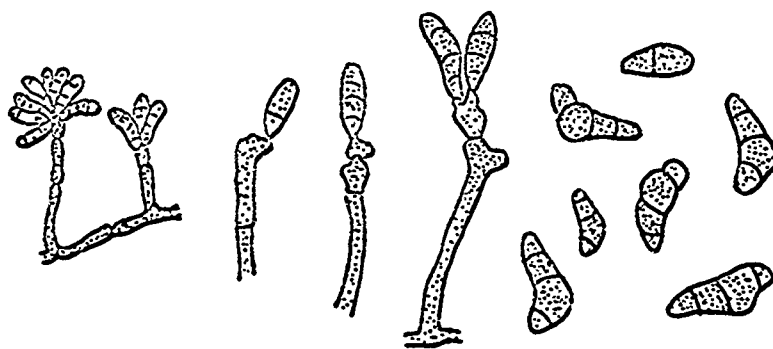
Cephalosporium



Pestalotia



Chalaropsis



Curcularia

- END -